National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation of Risks To Human Reproduction

NTP-CERHR MONOGRAPH ON THE POTENTIAL HUMAN REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF HYDROXYUREA

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PREFACE

The National Toxicology Program (NTP)[1] established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the CERHR is to provide timely, unbiased, scientifically sound evaluations of the potential for adverse effects on reproduction or development resulting from human exposures to substances in the environment. The NTP-CERHR is headquartered at the National Institute of Environmental Health Sciences (NIEHS) and Dr. Michael Shelby is the director.[2]

CERHR broadly solicits nominations of chemicals for evaluation from the public and private sectors. Chemicals are selected for evaluation based on several factors including the following:

- potential for human exposure from use and occurrence in the environment
- extent of public concern
- production volume
- extent of database on reproductive and developmental toxicity studies

CERHR follows a formal process for review and evaluation of nominated chemicals that includes multiple opportunities for public comment. Briefly, CERHR convenes a scientific expert panel that meets in a public forum to review, discuss, and evaluate the scientific literature on the selected chemical. CERHR expert panels use explicit guidelines to evaluate the scientific literature and prepare the expert panel reports. Public comment is invited prior to and during the meeting. The expert panel produces a report on the chemical's reproductive and developmental toxicities and provides its opinion of the degree to which exposure to the chemical is hazardous to humans. The panel also identifies areas of uncertainty and where additional data are needed. Expert panel reports are made public and comments are solicited.

Next, CERHR prepares the NTP Brief. The goal of the NTP Brief is to provide the public, as well as government health, regulatory, and research agencies, with the NTP's conclusions regarding the potential for the chemical to adversely affect human reproductive health or children's development. CERHR then prepares the NTP-CERHR Monograph, which includes the NTP Brief and the Expert Panel Report. The NTP-CERHR Monograph is made publicly available on the CERHR web site and in hardcopy or CD from CERHR.

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[1]NTP is an interagency program headquartered in Research Triangle Park, NC at the National Institute of Environmental Health Sciences, a component of the National Institutes of Health.

[2]Information about the CERHR is available on the web at *http://cerhr.niehs.nih.gov* or by contacting:

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ABSTRACT

NTP-CERHR MONOGRAPH ON THE POTENTIAL HUMAN REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF HYDROXYUREA

The National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) conducted an evaluation of the potential for hydroxyurea to cause adverse effects on reproduction and development in humans. Hydroxyurea is a drug used to treat cancer, sickle cell disease, and thalassemia. It is the only treatment for sickle cell disease in children, aside from blood transfusion and, in severe cases, hematopoietic stem cell transplantation. Hydroxyurea is FDA-approved for use in adults with sickle cell anemia to reduce the frequency of painful crises and the need for blood transfusions. Hydroxyurea may be given to children and adults with sickle cell disease for an extended period of time or for repeated cycles of therapy. Treatment with hydroxyurea is associated with known side effects such as cytotoxicity and myelosuppression, and hydroxyurea is genotoxic (can damage DNA).

CERHR selected hydroxyurea for evaluation because of:

- its increasing use for treatment of sickle cell disease in children and adults,
- knowledge that it inhibits DNA synthesis and is cytotoxic, and
- published evidence of reproductive and developmental toxicity in rodents.

The results of this evaluation are published in the NTP-CERHR Monograph on Hydroxyurea, which includes the NTP Brief and Expert Panel Report on the Reproductive and Developmental Toxicity of Hydroxyurea. Additional information related to the evaluation process, including public comments received on the draft NTP Brief and the final expert panel report, are available on the CERHR website (*http://cerhr.niehs.nih.gov/)*. See hydroxyurea under "CERHR Chemicals" on the homepage or go directly to *http://cerhr.niehs.nih.gov/chemicals/hydroxyurea/hydroxyurea-eval.html*).

The NTP reached the following conclusions on the possible effects of exposure to hydroxyurea on human reproduction or development. The possible levels of concern, from lowest to highest, are *negligible concern*, *minimal concern*, *some concern*, *concern*, and *serious concern*.

The NTP expresses *serious concern* that exposure of men to therapeutic doses of hydroxyurea may adversely affect sperm production. This level of concern is for all males who have reached puberty.

The NTP concurs with the Expert Panel that there is *concern* that exposure of pregnant women to hydroxyurea may result in birth defects, abnormalities of fetal growth, or abnormal postnatal development in offspring.

The NTP concurs with the Expert Panel that there is *minimal concern* that exposure of children to therapeutic doses of hydroxyurea at 5–15 years of age will adversely affect growth.

NTP will transmit the NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Hydroxyurea to federal and state agencies, interested parties, and the public and make it available in electronic PDF format on the CERHR web site *(http://cerhr.niehs.nih.gov)* and in printed text or CD from CERHR:

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INTRODUCTION

In October 2005, the CERHR Core Committee, an advisory committee composed of representatives from NTP member agencies, recommended hydroxyurea for expert panel evaluation. Hydroxyurea (CAS RN: 127-07-01) is a drug used to treat cancer, sickle cell disease, and thalassemia. It is the only treatment for sickle cell disease aside from blood transfusion and, in severe cases, hematopoietic stem cell transplantation which remains experimental in adults. Hydroxyurea is approved by the U.S. Food and Drug Administration (FDA) for use in adults with sickle cell anemia to reduce the frequency of moderate to severe painful crises and the need for blood transfusions. Treatment of children with sickle cell disease is currently an "off-label" use of hydroxyurea. (The term "off-label" refers to the legal use of a prescription drug to treat a disease or condition other than that for which the FDA has approved the drug. In the case of hydroxyurea, FDA has not approved its use in children.) It may be given to children and adults with sickle cell disease for an extended period of time or for repeated cycles of therapy. Treatment with hydroxyurea is associated with known side effects including cytotoxicity (toxicity to cells) and myelosuppression (reduced production of blood cells), and hydroxyurea can damage DNA (is genotoxic).

CERHR selected hydroxyurea for expert panel evaluation because of:

- its increasing use for treatment of sickle cell disease in children and adults,
- knowledge that it inhibits DNA synthesis and is cytotoxic, and
- published evidence of reproductive and developmental toxicity in rodents.

As part of its evaluation, CERHR convened a panel of scientific experts (Appendix I) to review, discuss, and evaluate the scientific evidence on the potential reproductive and developmental toxicities of hydroxyurea. A public meeting of the NTP-CERHR Hydroxyurea Expert Panel was held on January 24–26, 2007, in Alexandria, VA.

This monograph includes the NTP Brief on Hydroxyurea, a list of the expert panel members (Appendix I), and the Expert Panel Report on Hydroxyurea (Appendix II). The monograph is intended to serve as a single, collective source of information on the potential for hydroxyurea to adversely affect human reproduction or development. Those interested in reading this monograph may include individuals, members of public interest groups, staff of health and regulatory agencies and the medical and scientific communities.

The NTP Brief on Hydroxyurea presents the NTP's opinion on the potential for exposure to hydroxyurea to cause adverse reproductive or developmental effects in people. The NTP Brief is intended to provide clear, balanced, scientifically sound information. It is based on information about hydroxyurea provided in the expert panel report, public comments, comments from peer reviewers[3] and additional scientific information available since the expert panel meeting.

[3]Peer review of this brief was conducted by letter review. Reviewers were:

- John DeSesso, Ph.D.
  Noblis
- Jack Favor, Ph.D.
  Institute of Human Genetics, Munich
- Charles Peterson, M.D.
  National Heart, Lung, and Blood Institute
- Joe Rutledge, M.D.
  University of Washington

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NTP BRIEF ON HYDROXYUREA

[CAS RN: 127–07–1]

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NTP BRIEF ON HYDROXYUREA

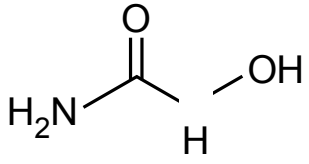
WHAT IS HYDROXYUREA?

Hydroxyurea (CAS RN: 127-07-1) is a prescription medicine approved by the FDA for treatment of adults with certain types of cancer and sickle cell disease. Off-label uses include treatment for various myeloproliferative disorders (such as leukemia), thalassemia, psoriasis, HIV infection, and sickle-cell disease in children. It is the only treatment for sickle cell disease aside from blood transfusion and, in severe cases, hematopoietic stem cell transplantation which remains experimental in adults. Treatment with hydroxyurea in children and adults with sickle cell disease may occur for an extended period of time, sometimes for years. Hydroxyurea treatment is associated with known side effects including cytotoxicity (toxicity to cells) and myelosuppression (reduced production of blood cells in the bone marrow), and genotoxicity (damage to DNA).

Sickle-cell disease can lead to painful vaso-occlusive crises where the sickle-shaped red blood cells obstruct capillaries and restrict blood flow to an organ or tissue, resulting in reduced blood supply, and potential organ damage. Hydroxyurea is FDA-approved for use in adults with sickle cell anemia who experience moderate to severe crises (generally ≥ 3 in the previous 12 months) to reduce the frequency of these crises and the need for blood transfusions.

Hydroxyurea is a virtually tasteless, white crystalline powder with a chemical formula of $CH_4N_2O_2$ (Figure 1).

Figure 1.
Chemical Structure of Hydroxyurea
($CH_4N_2O_2$; Molecular Weight 76.06)



Although treatment of children with sickle cell disease is currently an off-label use of hydroxyurea, usage in children is reported frequently and appears to be increasing. Hydroxyurea is the preferred therapy among families who have children with severe sickle cell anemia compared to other therapeutic options such as chronic red blood cell transfusion *(1)*. The National Institute of Child Health and Human Development and the National Heart, Lung, and Blood Institute of the National Institutes of Health are currently co-sponsoring a clinical trial to determine if hydroxyurea is effective for preventing chronic end-organ damage in infants and children with sickle cell disease.

The mechanisms by which hydroxyurea relieves the symptoms of sickle cell disease are not completely understood. However, it is known that sickle cell disease is less severe in individuals who produce high levels of fetal hemoglobin (hemoglobin F).[4] For many patients, hydroxyurea increases the production of hemoglobin F which helps prevent the formation of sickle-shaped red blood cells. In addition, hydroxyurea therapy can help prevent the vaso-occlusive crisis by improving movement of sickle-shaped red blood cells through the circulatory system.

Studies in cultured cells and animals show that hydroxyurea can damage DNA. It impairs the ability of cells to replicate DNA during the synthesis phase (S-phase) of the cell cycle. This impairment of cell division is the primary basis for its use in cancer chemotherapy.

Hydroxyurea, also commonly referred to as

[4]Hemoglobin is the iron-containing oxygen-transport protein found in red blood cells. Hemoglobin F is the main hemoglobin produced by the fetus in the second half of pregnancy. All adults produce small amounts of hemoglobin F.

hydroxycarbamide, is marketed under the names Hydrea® and Droxia® by Bristol-Myers Squibb. Companies that are FDA-approved to manufacture unbranded (generic) hydroxyurea include Barr Pharmaceuticals, Duramed Pharmaceuticals, Par Pharmaceuticals, and Roxane Laboratories. Information on the production volume of hydroxyurea in the United States is not available.

HOW ARE PEOPLE EXPOSED TO HYDROXYUREA?

People are exposed to hydroxyurea through prescribed medication. Recommended doses of hydroxyurea for adults range from 15–35 mg/kg bw/day depending on the specific disease and the patient's response to treatment. In children, starting doses of 10–20 mg/kg bw/day and maximum doses of 25–35 mg/kg bw/day have been reported. Although not approved by the FDA, the use of hydroxyurea in children has been reported frequently and appears to be increasing.

Hydroxyurea is not recommended for use during pregnancy because of concern for effects on the fetus. However, fetuses may be exposed if women conceive while on therapy. It is not known how many pregnant or nursing women are exposed to hydroxyurea. Hydroxyurea crosses the placenta and is found in breast milk. Thus, taking hydroxyurea during pregnancy or lactation exposes the unborn child or infant to this drug.

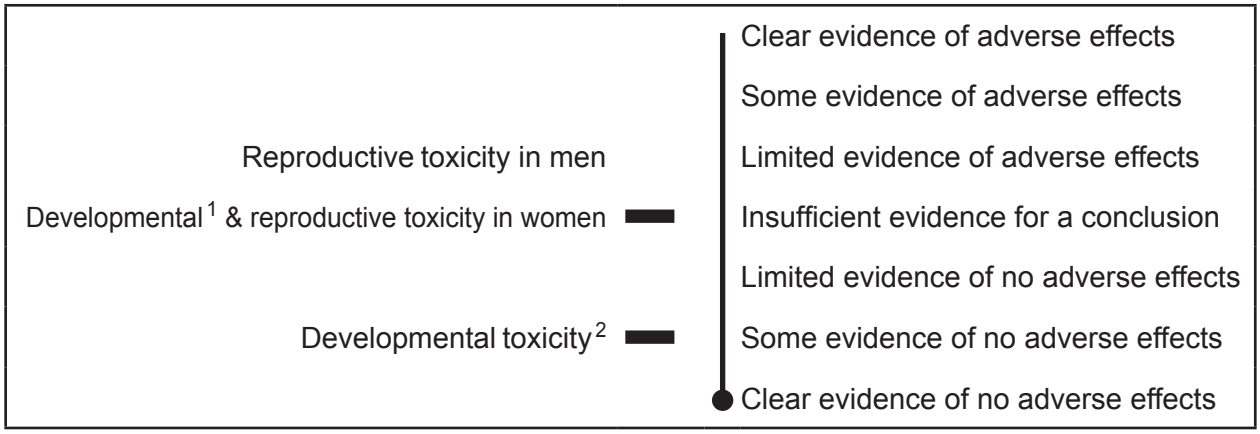
No information is available on occupational exposures associated with the manufacture, packaging, or distribution of hydroxyurea in the United States although it has been detected in the air in some European pharmaceutical workplaces *(2)*. No information is available on the occurrence of hydroxyurea in the environment.

CAN HYDROXYUREA AFFECT HUMAN DEVELOPMENT OR REPRODUCTION?[5]

Probably. In humans, there is no direct evidence that exposure to hydroxyurea adversely affects development but there is limited evidence of impaired reproductive function (decreased sperm count in some patients) (see Figure 2a). However, studies in laboratory animals show that exposure to hydroxyurea can cause adverse

[5]Answers to this and subsequent questions may be: *Yes, Probably, Possibly, Probably Not, No or Unknown*

Figure 2a. *The weight of evidence that hydroxyurea causes adverse developmental or reproductive effects in humans*



[1]For fetuses/infants of pregnant/breastfeeding women and children less than 5 years of age

[2]For children ages 5 to 15 years (based on growth assessments)

effects on development and on the male reproductive tract (see Figure 2b). In laboratory rodents, hydroxyurea produces birth defects, reduced numbers of live births, and abnormalities of fetal growth. In addition, experimental animal data show decreased testis weight and histologic abnormalities of seminiferous tubules in rats and mice, and decreased sperm counts in mice. The blood concentrations associated with some of these effects in laboratory animal studies are estimated to be similar to blood concentrations in patients on hydroxyurea therapy (Table 1).

Scientific decisions concerning health risks are generally based on what is known as the "weight-of-evidence." In this case, the NTP recognizes the lack of sufficient data on the effects of hydroxyurea in humans and the clear evidence of adverse effects in laboratory animals and judges the scientific evidence sufficient to conclude that hydroxyurea may adversely affect human development and reproduction if exposures are sufficiently high (see Figure 3). The NTP recognizes that hydroxyurea is used to treat serious illnesses and that the decision to

Figure 2b. *The weight of evidence that hydroxyurea causes adverse developmental or reproductive effects in laboratory animals*

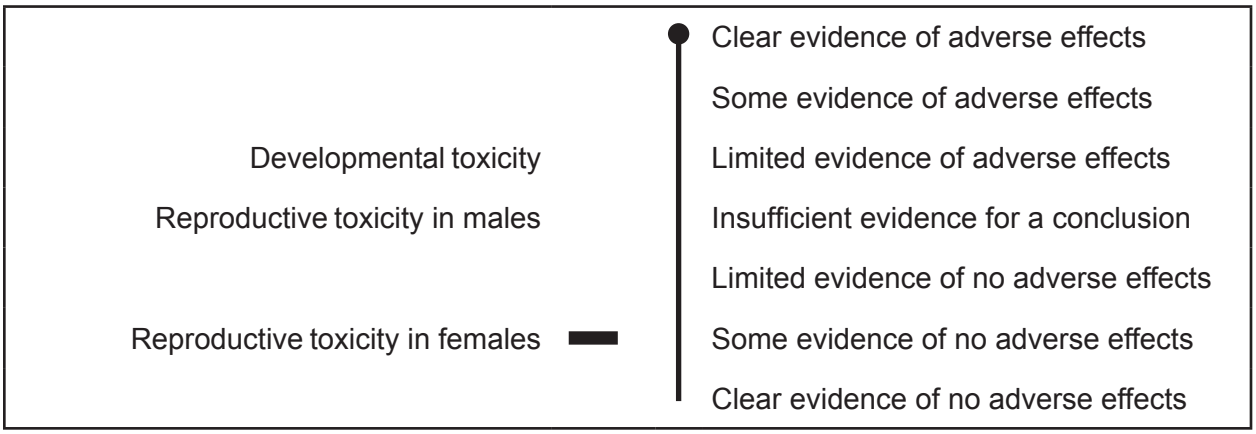
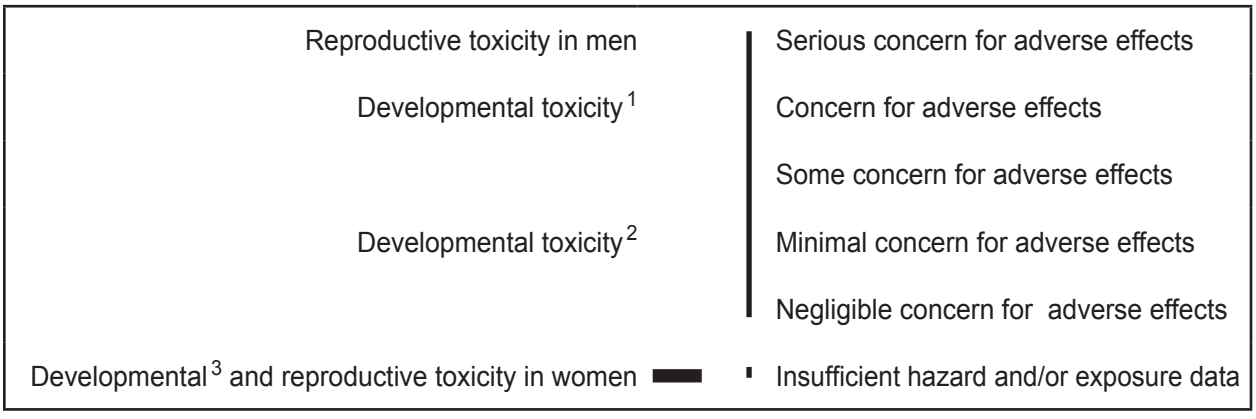


Figure 3. *NTP conclusions regarding the possibilities that human development or reproduction might be affected by exposure to hydroxyurea*



[1] For fetuses

[2] For growth and development in children 5-15 years of age

[3] For infants and children under 5 years of age

Table 1. *Comparison of Hydroxyurea Plasma Concentrations in Humans on Hydroxyurea Therapy to Concentrations in Mice at Dose Levels Associated with Developmental or Reproductive Toxicity*

Exposure	*Animal Model or Human Population*	*N*	*Sickle-Cell Disease*	*Renal Function*	*C_{max} or "peak" concentration (mg/L)*	*Reference*
Pharmacokinetic Studies in Mice (Intraperitoneal, mg/kg)						
100[a]	BALB/c nude	5			111	*(10)*
100[a]	C57BL/6	5			175	*(9)*
50[b]	C57BL/6	4			74	*(9)*
Therapeutically Relevant Doses for Humans (Oral, mg/kg/day)[c]						
29	Healthy men	24-30	–		48–51.9	*(13, 14)*
25	Men and women	6	+		21–54	*(15)*
20 *Range: 15 - 35*	Men and women	6	+		26.5	*(16)*
22 *Range: 14 - 37*	Girls and boys	11	+		24.5	*(16)*
15	Men and women	7	+	Normal	28.3	*(17)*
	Men and women	2-3 per category	+	Mild to severe impairment	22.0–28.8	*(17)*

[a]Developmental toxicity (external malformations and altered behavior) was observed in rats treated with 100 mg/kg/day intraperitoneal *(7)*

[b]Reproductive toxicity (decreased testis weight and altered distribution of testicular germ cells) was observed in mice treated with 50 mg/kg/day intraperitoneal *(18)*.

[c]Recommended starting doses of 10-20 mg/kg bw/day and maximum doses of 25-35 mg/kg bw/day have been used with adults and children with sickle cell disease. Higher doses are recommended for treating solid tumors.

use hydroxyurea is made by the patient and his or her clinician.[6]

SUPPORTING EVIDENCE

The expert panel report provides additional details and citations regarding studies on the possible reproductive and developmental toxicity of hydroxyurea [see Appendix II or *(3)*]. The expert panel evaluated several case reports and case series that described outcomes in a total of 58 pregnancies for 57 women exposed to hydroxyurea during gestation. One case of fetal death and several cases of minor malformations or reduced growth were reported, but the expert panel determined there was insufficient evidence to conclude that hydroxyurea causes or does not cause developmental toxicity when exposure occurs prenatally or during lactation *(3)*. The panel noted that hydroxyurea is used to treat serious illnesses during pregnancy, such as sickle cell disease and essential thrombocythemia, which can themselves affect pregnancy outcome. For this reason, it is difficult to separate possible hydroxyurea-induced adverse effects

[6]As noted by the Expert Panel, because children and young adolescents may not have the maturity to make informed decisions about reproductive health-related matters, the Expert Panel recognizes that clinicians caring for these children will involve parents, guardians, or other adults in some of these decisions. The NTP recognizes that some states require involvement of parents in reproductive health-related decisions affecting minor children.

from effects resulting from the disease itself. Several studies have evaluated growth (height and weight) and delays in development, such as onset of puberty, in children aged 5 to 15 years old. Although no growth or pubertal effects were reported in these studies, the panel noted that the durations of follow-up were relatively short, ranging from 6 months to 12 years. There were insufficient data for the panel to evaluate possible growth effects in children less than 5 years of age. Data were also not available on the long-term health effects, including abnormal development, impaired reproductive function, and risk of cancer, following fetal, childhood, or adolescent exposure to hydroxyurea.

There were sufficient experimental animal data available for the expert panel to conclude that hydroxyurea is a developmental toxicant following both oral and intraperitoneal routes of administration. In rats, hydroxyurea caused malformations, decreased fetal weight, and a decrease in the number of live pups at oral doses of 200 mg/kg bw/day during days 7 to 20 of gestation *(4, 5)* or ~300 mg/kg bw/day during days 6 to 15 of gestation *(6)*. Increases in malformations of the eye and head and altered behavior were observed at a lower administered dose when rats were exposed to hydroxyurea by intraperitoneal injection (100 mg/kg bw/day from gestational days 9 to 12) *(7)*. Similarly, hydroxyurea caused malformations, decreased body weight, increased resorptions and stillbirths in mice when dams were treated orally with 200 mg/kg bw/day during days 6 to 17 of gestation *(8)*. The malformations most commonly reported in rats and mice are neural tube defects, cleft palate, vertebral abnormalities, and deformities of the toes, such as polydactyly (extra digits), oligodactyly/adactyly (missing digits), syndactyly (webbed digits) or ectrodactyly ("lobster claw" syndrome). Mechanistic studies in rodents suggest the developmental toxicity of hydroxyurea relates to its ability to inhibit DNA synthesis with consequent arrest of the cell cycle and cell death.

The data were insufficient for the expert panel to evaluate the long-term effects of hydroxyurea in experimental animals exposed during gestation or as immature animals.

In reaching conclusions about possible developmental effects of hydroxyurea in humans, the NTP considered how the doses used in the laboratory studies relate to human exposures (see Table 1). Blood levels of hydroxyurea in people taking this medication are similar to blood levels in mice administered an intraperitoneal dose of hydroxyurea, 100 mg/kg, that is associated with adverse developmental effects (malformations of the eye and head and altered reflex response and behavior) *(7, 9, 10)*. Although human exposure is oral, the expert panel considered studies in laboratory animals that used intraperitoneal routes of administration as corroborative. This conclusion was primarily based on the finding that in mice similar amounts of hydroxyurea are excreted in the urine (~90%) within 24-hours following either oral or intraperitoneal dosing with 200 mg/kg *(11)* suggesting that absorption is roughly comparable between the two routes of administration. The NTP notes greater differences in 24-hour urinary excretion in rats based on route of administration (57% following oral dosing of 50 mg/kg and 90% after intraperitoneal administration of 100 mg/kg) *(11)*. The NTP would have more confidence in the comparability of the oral and intraperitoneal routes of administration in rodents if well-designed pharmacokinetic studies for oral administration were available. Despite this research gap the NTP concurs with the expert panel that intraperitoneal studies in laboratory animals are useful in evaluating potential risks to humans on hydroxyurea therapy. In addition, in humans, oral and intravenous administration of hydroxyurea produce similar pharmacokinetic profiles suggesting minimal impact of first-pass metabolism following oral delivery *(12)*.

The NTP compared measured peak plasma con-

centrations in mice treated intraperitoneally with 100 mg/kg hydroxyurea to peak plasma concentrations in humans following oral administration of clinically relevant doses of hydroxyurea (Table 1) *(3)*. The NTP considers it reasonable to use a mouse pharmacokinetic study to estimate blood levels in the rat treated with an intraperitoneal dose of 100 mg/kg/day because the rat and mouse excrete similar amounts hydroxyurea in urine (24-hour urinary excretion of ~90% following intraperitoneal administration of 200 mg/kg in mice and 100 mg/kg in rats) and have similar half-lives of 11-15 minutes following intraperitoneal dosing with 100 mg/kg *(3, 11)*.

Peak plasma concentrations in mice at 100 mg/kg was 111 mg/L in BALB/c nude mice and 175 mg/kg in C57BL/6 mice *(9, 10)*, which is approximately 2 to 8 times higher than peak plasma concentrations in humans following treatment with 25 or 29 mg/kg hydroxyurea. (Table 1). In addition, data from rat and monkey studies indicate that hydroxyurea is eliminated more slowly from the embryo compared to the mother and that concentrations of hydroxyurea in the embryo exceed concentrations in maternal plasma shortly after exposure *(3*). Finally, estimates based on a pharmacokinetic model show the same average concentration of hydroxyurea in rat embryos whose mothers were dosed with 100 mg/kg bw/day during gestation as human embryos whose mothers took 10 mg/kg bw/day (69 mg-hour/L) *(3)*. Although the assumptions and conclusions of this model need to be verified before it can be applied to risk prediction, it supports NTP's concern for hydroxyurea usage during pregnancy.

Although some clinical reports of impaired reproductive function in adult men are available, no studies with sufficient sample size were available to the expert panel to evaluate possible reproductive effects of hydroxyurea in people treated during childhood, adolescence, and/or adulthood. A clinical report reviewed by the expert panel presented the case of a 27-year old man with a sperm count of zero (azoospermia) after 6-months of hydroxyurea treatment. Prior to beginning hydroxyurea treatment his sperm count was normal (88 million/ml) and rebounded to a low-normal concentration (35 million/ml) within a year of discontinuing hydroxyurea treatment. This study was not considered useful for the expert panel evaluation because it included results from only one person. However, subsequent to the expert panel meeting, two additional studies were published that presented case reports where hydroxyurea appeared to inhibit spermatogenesis in some male patients. In one study, a 35-year old man on hydroxyurea treatment for 3 years was diagnosed with infertility and azoospermia. Within 6 months of stopping hydroxyurea, the patient's sperm levels returned to normal and his wife conceived *(19)*. In another study, a 27-year old man was azoospermatic during hydroxyurea treatment but had low-normal sperm count (30 million/ml) following cessation of hydroxyurea for 3 months. He again became azoospermatic when hydroxyurea treatment was re-initiated. Another patient did not show a decrease in sperm count while taking hydroxyurea (26 million/ml during treatment and 15 million/ml after ending treatment) *(20)*. Thus, while some of the case reports support experimental animal data in mice of decreased sperm counts *(21)*, it appears there may be considerable variation in responses among men. Detecting the impacts of hydroxyurea on human sperm count and function is complicated because sperm abnormalities are associated with untreated sickle cell disease. Inhibition of DNA synthesis and cell cycle arrest by hydroxyurea offers a possible mechanism for its impact on sperm counts.

The available experimental animal data are too limited to completely evaluate the effects of hydroxyurea on fertility and reproduction, especially for females. The existing data show that hydroxyurea produces reproductive toxic-

ity in male rats at ~400–460 mg/kg bw/day in drinking water for 70–90 days as manifested by reduced testis weight and histologic abnormalities of seminiferous tubules. In male mice, intraperitoneal (ip) injection of 50 mg/kg bw/day hydroxyurea for 5 days caused decreased testis weight and flow cytometric abnormalities in testicular germ cell distribution. In male mice, higher ip doses of 625 to 5000 mg/kg bw/day hydroxyurea decreases sperm count 38 to 79 percent.

In reaching conclusions about reproductive effects of hydroxyurea, the NTP considered how the doses used in the laboratory studies relate to human exposures (Table 1). Blood levels of hydroxyurea in people taking therapeutic doses are similar to blood levels in mice administered an intraperitoneal dose of hydroxyurea, 50 mg/kg, that is associated with adverse reproductive effects (decreased testis weight and flow cytometric abnormalities in testicular germ cell distribution in mice) *(9, 10, 18)*. This conclusion is primarily based on comparing the measured peak plasma concentration in mice treated intraperitoneally with 50 mg/kg hydroxyurea to peak plasma concentrations in humans following oral administration of clinically relevant doses of hydroxyurea. In this study, the measured peak plasma concentration in C57/B6 mice at 50 mg/kg *(9)* is approximately 1.4 to 3.5 times higher than peak plasma concentrations in humans following treatment with 25 or 29 mg/kg hydroxyurea, i.e., 74 mg/L versus 21-54 mg/L in adults with sickle-cell disease *(15*) and 48-52 mg/L in healthy adults *(13, 14)*.

Several additional studies involving hydroxyurea treatment in humans have been published subsequent to the expert panel review. However, these studies either did not assess or did not report information related to evaluating developmental or reproductive hazard of hydroxyurea *(19, 22-32)*. One study reported higher cognitive function (verbal comprehension, fluid reasoning, and general cognitive ability) in children on hydroxyurea compared to those not on hydroxyurea treatment, possibly due to improved blood and oxygen supply to the brain or decreased fatigue and illness *(33)*. The NTP did not identify any additional developmental or reproductive animal toxicity studies published in the peer-reviewed literature subsequent to the expert panel evaluation.

SHOULD EXPOSURES TO HYDROXYUREA CAUSE CONCERN?

Yes. Clinical reports indicate that hydroxyurea can impair sperm production in some males. The NTP considers these reports of decreased sperm production in some men on hydroxyurea therapy to be consistent with the expert panel's determination from studies in laboratory animals that hydroxyurea causes decreased testis weight and sperm counts, as well as histologic abnormalities of seminiferous tubules. It is not known if the effects in laboratory animals resulted in impaired reproductive function because fertility was not assessed in these studies. Despite the magnitude of the effects on sperm count in mice, e.g., reductions of 38 to 79% for intraperitoneal doses ranging from 625 to 5000 mg/kg bw/day, it is not clear that fertility in these animals would have been impacted because sperm count decrements of ~80% do not necessarily lead to reductions in fertility in laboratory rodents. Laboratory rodents are generally considered to be hyperfertile compared to men; therefore, decreases in sperm counts that do not necessarily impair fertility in rodents may correspond to an adverse effect in humans *(34)*. In addition, clinical reports of azoospermia and decreases in sperm count corresponding to periods of hydroxyurea usage (and rebounding to normal levels during discontinuation of treatment) suggest that, at least in some men, hydroxyurea can have significant effects on sperm count and adversely affect fertility. Blood levels of hydroxyurea in people taking this medication are similar to blood levels in

mice administered a dose of hydroxyurea, 50 mg/kg, that is associated with a adverse reproductive effects (decreased testis weight and flow cytometric abnormalities in testicular germ cell distribution in mice) *(9, 10, 18)*.

In February 2008, the National Institutes of Health (NIH) sponsored a Consensus Development Conference on Hydroxyurea Treatment for Sickle Cell Disease. The consensus statement issued by this independent panel of scientists recognized the possibility for temporary decreases in sperm counts or sperm abnormalities as a side-effect of treatment. They concluded that the risks of such effects in adults are acceptable compared to the risk of untreated sickle cell disease *(35)*. Fertility management options for men can include (1) banking sperm prior to treatment, (2) annual monitoring of sperm counts, and (3) use of contraception during therapy and for at least 3 months after ending treatment *(20)*. Data are not sufficient in humans or experimental animals to evaluate possible reproductive effects in women on hydroxyurea therapy.

Hydroxyurea is not recommended for use during pregnancy *(35)*. However, fetuses may be exposed if women conceive while on hydroxyurea therapy. Although sufficient data are not available to determine if exposure to hydroxyurea during pregnancy adversely affects the human fetus, animal data from multiple species indicate that hydroxyurea produces malformations, reduced number of live births, and abnormalities in fetal growth. Blood levels of hydroxyurea in people taking this medication are similar to blood levels in mice administered a dose of hydroxyurea, 100 mg/kg, that is associated with adverse developmental effects in rats (malformations of the eye and head and altered reflex response and behavior) *(7, 9, 10)*.

There is no evidence from the studies reviewed that hydroxyurea treatment at therapeutic doses affects growth or development (i.e., pubertal progression) in children age 5 – 15 years. However, data are not sufficient to evaluate possible effects on growth and development in infants and children younger than 5 years.

NTP CONCLUSIONS

The NTP reached the following conclusions on the possible effects of exposure to hydroxyurea on human development and reproduction. Note that the possible levels of concern, from lowest to highest, are *negligible concern, minimal concern, some concern, concern,* and *serious concern.*

The NTP has *serious concern* that exposure of men to therapeutic doses of hydroxyurea may adversely affect sperm production. This level of concern is for all males who have reached puberty.

This level of concern is higher than that expressed by the NTP-CERHR Expert Panel and is based on (1) experimental animal data showing decreased testis weight and sperm count, as well as cellular effects on the testes, and (2) additional clinical reports of decreased or zero sperm count in men undergoing hydroxyurea therapy. These reports were not published when the expert panel completed its deliberations. Blood levels of hydroxyurea in people taking this medication are similar to blood levels in laboratory animals administered a dose of hydroxyurea that is associated with adverse reproductive effects. The *"serious concern"* expressed by the NTP for effects on sperm production does not necessarily conflict with conclusions reached by other scientists or panels who have concluded that such risks are acceptable compared to the risk of untreated sickle cell disease. The NTP recognizes that hydroxyurea is used to treat serious illnesses and that the decision to use hydroxyurea by a man of reproductive age is made by the patient and his clinician. Fertility management options for men can include (1) banking sperm prior to treatment, (2) annual monitoring of sperm counts, and (3) use of contraception during therapy and for at least 3 months after ending treatment.

The NTP has *concern* that exposure of pregnant women to hydroxyurea at therapeutic doses may result in birth defects or abnormalities of fetal growth and postnatal development in their offspring.

The NTP concurs with the NTP-CERHR Expert Panel that there is *concern* that exposure of pregnant women to hydroxyurea at therapeutic doses may result in birth defects or abnormalities of fetal growth and postnatal development in their offspring. This conclusion is based on data from animal studies showing that hydroxyurea produces birth defects, reduced number of live births, and abnormalities of fetal growth in multiple laboratory animal species. Blood levels of hydroxyurea in people taking this medication are similar to blood levels in laboratory animals administered a dose of hydroxyurea that is associated with adverse developmental effects. The current advice for women who are trying to become pregnant or become pregnant while taking hydroxyurea is to stop taking the drug. The NTP recognizes that hydroxyurea is used to treat serious illnesses and that the decision to use hydroxyurea by a woman of reproductive age or by a pregnant woman is made by the patient and her clinician.

The NTP has *minimal concern* that exposure of children to therapeutic doses of hydroxyurea at 5–15 years of age will adversely affect growth.

The NTP concurs with the NTP-CERHR Expert Panel that there is *minimal concern* that exposure of children to therapeutic doses of hydroxyurea at 5–15 years of age will adversely affect growth. This conclusion is based on human studies reporting no adverse effects on growth (height and weight) or development (i.e., onset of puberty). However, the absence of studies on

the long-term health effects, including effects on reproductive function and risk of cancer, following childhood exposures to hydroxyurea support expressing "minimal" concern as opposed to "negligible" concern. In addition, there are no data on growth effects in children less than 5 years of age. As noted above, there is serious concern for effects on spermatogenesis in all males who have reached puberty.

These conclusions are based on information available at the time this brief was prepared. As new information on toxicity and exposure accumulates, it may form the basis for either lowering or raising the levels of concern expressed in the conclusions.

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APPENDIX I. NTP-CERHR HYDROXYUREA EXPERT PANEL

A 13-member panel of scientists covering disciplines such as toxicology, epidemiology, and medicine was recommended by the CERHR Core Committee and approved by the Associate Director of the NTP. Prior to the expert panel meeting, the panelists critically reviewed articles from the scientific literature, as well as a variety of other relevant documents. Based on this material, they identified key studies and issues for discussion. At a public meeting held January 24–26, 2007, the expert panel discussed these studies, the adequacy of available data, and identified data needed to improve future assessments. The expert panel reached conclusions on whether exposure to hydroxyurea might result in adverse effects on human reproduction or development. Panel conclusions were based on the scientific evidence available at the time of the public meeting. The NTP-CERHR released the final expert panel report for public comment on March 5, 2007 and the deadline for public comments was April 18, 2007 *(Federal Register Vol. 72:8384–8385, 2007)*. The expert panel report on hydroxyurea is provided in Appendix II. The expert panel report is also available on the CERHR website (*http://cerhr.niehs.nih.gov*).

NTP-CERHR HYDROXYUREA EXPERT PANEL

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Edward Stanek III, Ph.D. University of Massachusetts Amherst, MA	

APPENDIX II. EXPERT PANEL REPORT ON HYDROXYUREA

The NTP-CERHR Expert Panel Report on Hydroxyurea is available in several formats:

CERHR website:
PDF: *http://cerhr/chemicals/hydroxyurea/hydroxyurea_final.pdf* or on the

Birth Defects Research journal website:
HTML: *http://www3.interscience.wiley.com/cgi-bin/fulltext/115806235/HTMLSTART*
PDF: *http://www3.interscience.wiley.com/cgi-bin/fulltext/115806235/PDFSTART*

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Expert Panel Report

NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Hydroxyurea

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PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of CERHR is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction and development caused by agents to which humans may be exposed.

Hydroxyurea was selected for evaluation by a CERHR expert panel because of (1) its increasing use in the treatment of sickle cell disease in children and adults, (2) knowledge that it inhibits DNA synthesis and is cytotoxic, and (3) published evidence of its reproductive and developmental toxicity in rodents. Hydroxyurea is FDA-approved for reducing the frequency of painful crises and the need for blood transfusions in adults with sickle cell anemia who experience recurrent moderate-to-severe crises. Hydroxyurea is used in the treatment of cancer, sickle cell disease, and thalassemia. It is the only treatment for sickle cell disease aside from blood transfusion used in children. Hydroxyurea may be used in the treatment of children and adults with sickle cell disease for an extended period of time or for repeated cycles of therapy. Treatment with hydroxyurea may be associated with cytotoxic and myelosuppressive effects, and hydroxyurea is mutagenic.

To obtain information about hydroxyurea for the CERHR evaluation, the PubMed (Medline) and Toxline databases were searched through January 10, 2007 using ''hydroxyurea'' and its CAS RN (127–07–1). References were also identified from databases such as REPROTOX, HSDB, IRIS, and DART and from the bibliographies of reports being reviewed.

This evaluation results from the efforts of a 13-member panel of government and non-government scientists that culminated in a public expert panel meeting held January 24–26, 2007. This report is a product of the hydroxyurea expert panel and is intended to (1) interpret the strength of scientific evidence that hydroxyurea is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures, especially for patients receiving hydroxyurea in the treatment of sickle cell disease and other health disorders, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future evaluations. This report has been reviewed by members of the hydroxyurea expert panel and by CERHR staff scientists. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating government agencies.

Dr. Skalko was unable to participate in the Expert Panel meeting but participated in the drafting and review of the report before and after the meeting.

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This Expert Panel Report will be included in the subsequent NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Hydroxyurea. That monograph will include the NTP-CERHR Brief, the Expert Panel Report, and all public comments on the Expert Panel Report. The NTP-CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, North Carolina, and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

Reports can be obtained from the web site (http://cerhr.niehs.nih.gov) or from: Michael D. Shelby, PhD, NIEHS EC-32, PO Box 12233, Research Triangle Park, NC 27709. E-mail: shelby@niehs.nih.gov

1.0 CHEMISTRY USE AND HUMAN EXPOSURE

1.1 Chemistry

1.1.1 Nomenclature. Hydroxyurea (127-07-1) synonyms listed in the ChemIDplus database (ChemIDplus, 2004) include: Biosupressin; Carbamohydroxamic acid; Carbamohydroximic acid; Carbamoyl oxime; Carbamyl hydroxamate; DRG-0253; Droxia; HU; Hidrix; Hidroxicarbamida [Spanish]; Hydrea; Hydroxicarbamidum; Hydroxycarbamide; Hydroxycarbamidum; Hydroxycarbamine; Hydroxyharnstoff [German]; Hydroxylamine, N-(aminocarbonyl)-; Hydroxylamine, N-carbamoyl-; Hydura; Hydurea; Idrossicarbamide; Litalir; N-Carbamoylhydroxylamine; N-Hydroxymocovina [Czech]; N-Hydroxyurea; Onco-Carbide; and Oxyurea. Hydroxyurea is marketed by Bristol-Myers Squibb under the names Hydrea (Bristol-Myers-Squibb, 2004) and Droxia (Bristol-Myers-Squibb, 2002).

1.1.2 Formula and molecular mass. The chemical formula for hydroxyurea is $CH_4N_2O_2$ (ChemIDplus, 2004). The molecular mass is 76.06 (FDA, 1998b). The structure for hydroxyurea is shown in Figure 1.

1.1.3 Chemical and physical properties. Hydroxyurea is a virtually tasteless, white crystalline powder (Bristol-Myers-Squibb, 2005b). Chemical and physical properties of hydroxyurea are listed in Table 1.

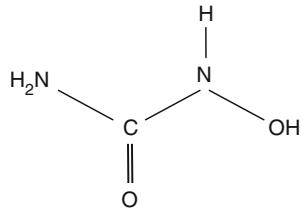

Fig. 1. Hydroxyurea structure.

Table 1
Chemical and Physical Properties of Hydroxyurea

Parameter	Value
Melting point	141°C
Log P (octanol-water)	−1.80
Water solubility	1.00×10^6 mg/L at 25°C
Vapor pressure	2.43×10^{-3} mm Hg at 25°C
Henry's law constant	5.42×10^{-11} atm-m^3/mol

From ChemIDplus (2004).

1.1.4 Technical products and impurities. Hydroxyurea is available as capsules or tablets (FDA, 2006). Capsules are available in strengths of 200, 300, 400, and 500 mg. The tablet is available in a 1000 mg strength. Inactive ingredients that may be present in capsules include citric acid, D&C Yellow #10, FD&C Blue #1, FD&C Red 40, D&C Red 28, D&C Red #33, FD&C Green #3, FD&C yellow #6, gelatin, lactose, magnesium stearate, sodium phosphate, titanium dioxide, silicon dioxide, or sodium lauryl sulfate (FDA, 1998a; Bristol-Myers-Squibb, 1999; 2001a,b; 2004; 2005a,b). No information was found about inactive ingredients in tablets.

1.2 Use and Human Exposure

1.2.1 Production information. Hydroxyurea has been produced by reacting calcium or potassium cyanate with hydroxylamine nitrate or hydroxylamine hydrochloride in absolute ethanol or aqueous solution (IARC, 2000). Another production method involves reacting a quaternary ammonium anion exchange resin with cyanate and then with hydroxylamine hydrochloride.

Bristol-Myers Squibb is the only company that manufactures branded hydroxyurea in the US. Companies that are Food and Drug Administration (FDA)-approved to manufacture unbranded (generic) hydroxyurea include Barr, Duramed Pharmaceuticals, Par Pharmaceuticals, and Roxane (FDA, 2006).

No information was located on U.S. production volume of hydroxyurea.

1.2.2 Use. Hydroxyurea is FDA-approved for reducing the frequency of painful crises and the need for blood transfusions in adults with sickle cell anemia who experience recurrent moderate-to-severe painful crises (generally $\geq$3 in the previous 12 months) (Bristol-Myers-Squibb, 2005a). **[Sickle cell anemia is discussed in Section 2.1.]**. Hydroxyurea is also FDA-approved as an anti-neoplastic agent in treatment of melanoma, resistant chronic myeloid leukemia, and recurrent metastatic or inoperable ovarian carcinoma (FDA, 1998a; Bristol-Myers-Squibb, 2005b). Concomitant use of hydroxyurea

Prepared With the Support of CERHR Staff: NTP/NIEHS, Michael Shelby, Ph.D. (Director, CERHR), Paul M.D. Foster, Ph.D. (Deputy Director, CERHR), Allen Dearry, Ph.D. (Interim Associate Director, NTP), Mary Wolfe, Ph.D. (NTP Liaison and Scientific Review Office); Sciences International, Inc., Anthony Scialli, M.D. (Principal Scientist), Annette Iannucci, M.S. (Toxicologist), Gloria Jahnke, D.V.M. (Toxicologist), Elizabeth Cho-Fertikh, Ph.D. (Toxicologist), Vera Jurgenson, M.S. (Research Associate).This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available on the CERHR web site (http://cerhr.niehs.nih.gov/). The format for Expert Panel Reports includes synopses of studies reviewed, followed by an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the Panel often makes comments or notes limitations in the synopses of the study. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from those of the authors, and conversions or analyses of data conducted by the Panel.**The findings and conclusions of this report are those of the expert panel and should not be construed to represent the views of the National Toxicology Program**.

with radiation therapy to control squamous cell (epidermoid) cancers of the head and neck, not including the lip, is also approved by the FDA (FDA, 1998a; Bristol-Myers-Squibb, 2005b).

According to a review by Gwilt and Tracewell (1998), primary chemotherapeutic uses of hydroxyurea include treatment of myeloproliferative disorders such as leukemia and polycythemia vera. Because of low response, hydroxyurea is not part of the standard chemotherapy of melanoma, ovarian cancer, squamous cell cancers of the head and neck, kidney cell, transitional carcinoma of urinary bladder, and prostate. Hydroxyurea has been used as a radiosensitizing agent for some malignancies and may be particularly useful as such in treating advanced carcinoma of the uterine cervix.

Treatment of children with sickle cell disease is an off-label use of hydroxyurea (Ohene-Frempong and Smith-Whitley, 1997). Hydroxyurea is being investigated currently for use in children as young as 6 months old with sickle cell disease to prevent chronic end-organ damage (NHLBI, 2007). Other reported off-label uses of hydroxyurea include treatment of psoriasis and human immunodeficiency virus infection (Gwilt and Tracewell, 1998).

Zumberg et al. (2005) surveyed hematologists/oncologists in North Carolina and Florida about their use of hydroxyurea in adults with sickle cell disease. In 2002, there were 342 eligible physicians who responded to the survey and 335 questionnaires that yielded sufficient data for analysis. Of the respondents completing those questionnaires, 58% practiced in communities, 30% practiced in university hospitals, and 12% practiced in university-affiliated institutions. Among the 166 community-based physicians, 43% saw fewer than one sickle cell disease patient per month, 74% saw less than two per month, 19% saw three to five per month, and 8% saw greater than six sickle cell disease patients per month. Among the community-based physicians, 45% prescribed hydroxyurea to $<10\%$ of sickle cell disease patients, 19% to 10–30% of patients, 20% to 31–60% of patients, 11% to 61–90% of patients, and 5% prescribed hydroxyurea to $>90\%$ of sickle cell disease patients. Indications for hydroxyurea among 161 community practitioners were greater than or equal to three painful crises per year (76%), use of narcotics for pain (58%), acute chest syndrome (43%), stroke history (40%), symptomatic severe anemia (31%), priapism (27%), low fetal hemoglobin (hemoglobin F) levels (29%), ankle ulcers (19%), renal failure (7%), pulmonary hypertension (7%), other disorders (e.g., thrombocytosis, need for frequent transfusions, cardiomyopathy) (5%), and elevated white cell count (3%). Patterns of hydroxyurea use by university-based practitioners were similar to those of community-based physicians, with the exception that university-based practitioners prescribed hydroxyurea more often for acute chest syndrome[1], stroke, and pulmonary hypertension.

1.2.3 Human exposure. Hydroxyurea is administered chronically, sometimes for years, for the treatment of sickle cell disease. For treatment of adults with sickle cell disease, hydroxyurea doses of 15–35 mg/kg bw/day are recommended (Bristol-Myers-Squibb, 2005a).

[1]Acute chest syndrome refers to pulmonary infiltrate or scan abnormality associated with infection, infarction, or both.

Table 2
Hematologic Values for Determining Appropriate Hydroxyurea Doses

Blood cell	Acceptable range[a]	Toxic range
Neutrophils (cells/mm^3)	≥ 2500	<2000
Platelets (mm^3)	$\geq 95{,}000$	$<80{,}000$
Hemoglobin (g/dL)	>5.3	<4.5
Reticulocytes (mm^3)	$\geq 95{,}000$	$<80{,}000$

From the product label (Bristol-Myers-Squibb, 2005a).
[a]For hemoglobin concentration <9 g/dL.

If hematologic testing shows acceptable blood count values (Table 2), the initial dose of 15 mg/kg bw/day may be increased by 5 mg/kg bw/day every 12 weeks until the maximum tolerated dose or maximum recommended dose of 35 mg/kg bw/day is obtained. An increase in dose is not recommended when blood counts are between acceptable and toxic ranges (Table 2). When blood counts are in the toxic range, hydroxyurea is discontinued until blood counts recover. On recovery, resumption of treatment is recommended with a dose reduction of 2.5 mg/kg bw/day. Up-or-down titration of the dose by increments of 2.5 mg/kg bw/day every 12 weeks is recommended until a dose that does not result in toxicity for 24 weeks is achieved. It is recommended that doses resulting previously in toxicity should not be administered again. A reduction in dose is recommended for patients with impaired renal function (Yan et al., 2005), and in patients with renal insufficiency, a starting dose of 7.5 mg/kg bw/day is recommended. A survey of oncologists/hematologists who treated sickle cell disease patients in Florida and North Carolina indicated that 62% of the physicians surveyed increased hydroxyurea doses until myelotoxicity was observed, 49% increased dosage until symptom relief was obtained, and 11% increased dosage to the recommended maximum level (Zumberg et al., 2005). When the physicians were asked what they believed to be the maximum hydroxyurea dose, responses ranged from 10–35 mg/kg bw (mean = 22 mg/kg bw).

Hydroxyurea is not recommended for use during pregnancy. However, pregnant women may be exposed if they conceive while on therapy. Approximately 760 deliveries/year occurred in women with sickle cell anemia between 1993–2004 (The National Inpatient Sample Database, Sophie Lanzkron, personal communication, January 26, 2007). These women are the ones most likely to be taking hydroxyurea at the time of pregnancy.

Although not approved by the FDA, the use of hydroxyurea in children has been reported frequently. In those reports, starting doses of 10–20 mg/kg bw/day and maximum doses of 25–35 mg/kg bw/day were reported for children (Ohene-Frempong and Smith-Whitley, 1997). Blood counts were monitored every 2–12 weeks, and intervals for dose increases were reported to be 4–12 weeks.

Hydroxyurea dose recommendations for treatment of solid tumors include 80 mg/kg bw every third day or 20–30 mg/kg bw/day (Bristol-Myers-Squibb, 2005b). In concomitant use with irradiation to treat solid tumors, the recommended hydroxyurea dose is 80 mg/kg bw every third day. It is suggested that hydroxyurea

treatment begin at least 1 week before commencement of irradiation and continue indefinitely if the patient can be adequately monitored and shows no evidence of severe reaction.

The recommended dose for treatment of resistant chronic myelocytic leukemia is 20–30 mg/kg bw/day (Bristol-Myers-Squibb, 2005b). Indefinite continuation of hydroxyurea therapy is recommended if there is regression of tumor size or arrest of tumor growth. If leukocyte counts drop below $2500/mm^3$ or platelet counts fall below 100,000 mm3, it is recommended that hydroxyurea therapy be stopped until white blood cell and platelet counts return to acceptable levels.

1.3 Utility of Exposure Data

Human exposure data include dose ranges for approved therapeutic uses of hydroxyurea. There is also information on dose ranges given to children, a use that is not approved by the FDA. There are no data on the number of children treated with hydroxyurea. Some information on blood levels after dosing of adults is available in Section 2. It is not known how many pregnant or nursing women are exposed to hydroxyurea, resulting in the exposure of the fetus or child. No information was identified on possible occupational exposure to hydroxyurea.

1.4 Summary of Human Exposure Data

Hydroxyurea is FDA-approved for reducing the frequency of painful crises and the need for blood transfusions in adults with sickle cell anemia who experience recurrent moderate-to-severe painful crises (generally ≥ 3 in the previous 12 months) (Bristol-Myers-Squibb, 2005a). A 2002 survey of 166 community-based hematologists/oncologists in North Carolina and Florida indicated that the majority of physicians (74%) saw fewer than two sickle cell disease patients each month (Zumberg et al., 2005). Of the physicians surveyed, 45% prescribed hydroxyurea to $<10\%$ of sickle cell disease patients, 19% to 10–30% of patients, 20% to 31–60% of patients, 11% to 61–90% of patients, and 5% prescribed hydroxyurea to $>90\%$ of sickle cell disease patients.

Hydroxyurea is administered chronically, sometimes for years, for the treatment of sickle cell disease. For treatment of sickle cell disease in adult patients, hydroxyurea doses of 15–35 mg/kg bw/day are recommended (Bristol-Myers-Squibb, 2005a). Drug labels recommend a starting dose of 15 mg/kg bw/day with increases of 5 mg/kg bw/day every 12 weeks until there is evidence of myelotoxicity or until the maximum recommended dose is reached. A survey of oncologists/hematologists who treated sickle cell disease patients in Florida and North Carolina showed that 62% of the physicians surveyed increased hydroxyurea doses until myelotoxicity was observed, 49% increased dosage until symptom relief was obtained, and 11% increased dosage to the recommended maximum level (Zumberg et al., 2005).

Although not approved by the FDA, the use of hydroxyurea in children has been reported frequently. Use in children appears to be increasing, and efficacy in younger children is being studied (NHLBI, 2007). In those reports, starting doses of 10–20 mg/kg bw/day and maximum doses of 25–35 mg/kg bw/day were used (Ohene-Frempong and Smith-Whitley, 1997). Blood counts were monitored every 2–12 weeks and intervals for dose increases were reported to be 4–12 weeks. There is no information available for hydroxyurea doses administered in other off-label uses, such as treatment of psoriasis and human immunodeficiency virus infection (Gwilt and Tracewell, 1998).

Hydroxyurea therapy is currently not recommended for pregnant women. Pregnancy exposures have occurred in women who became pregnant while on therapy. Approximately 760 deliveries/year occurred in women with sickle cell anemia between 1993–2004 (The National Inpatient Sample Database, Sophie Lanzkron, personal communication, January 26, 2007). These women are the ones most likely to be taking hydroxyurea at the time of pregnancy. Data on effects of hydroxyurea in pregnant women are discussed in Section 3.1.

Exposure of nursing infants may occur. Excretion of hydroxyurea in human milk is discussed in Section 2.2.1.2.

Hydroxyurea is FDA-approved as an anti-neoplastic agent in treatment of melanoma, resistant chronic myelocytic leukemia, and recurrent metastatic or inoperable ovarian carcinoma (FDA, 1998a; Bristol-Myers-Squibb, 2005b). The FDA also approved the use of hydroxyurea concomitantly with radiation therapy to control squamous cell (epidermoid) cancers of the head and neck, not including the lip (FDA, 1998a; Bristol-Myers-Squibb, 2005b). The primary chemotherapeutic uses of hydroxyurea were reported to be treatment of myeloproliferative disorders such as leukemia and polycythemia vera (Gwilt and Tracewell, 1998). Hydroxyurea dose recommendations for treatment of solid tumors include 80 mg/kg bw every third day or 20–30 mg/kg bw/day (Bristol-Myers-Squibb, 2005b). The recommended dose for treatment of resistant chronic myeloid leukemia is 20–30 mg/kg bw/day (Bristol-Myers-Squibb, 2005b). In chemotherapeutic applications, indefinite treatment is recommended if the treatment is found to be effective and the patient shows no evidence of severe toxicity.

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL EFFECTS

2.1 Pharmacodynamics, Normal Hemoglobin, and Sickle Cell Disease

Hydroxyurea inhibits the enzyme ribonucleotide reductase, which catalyzes the conversion of ribonucleotides to deoxyribonucleotides (Koç et al., 2004). The depletion of deoxyribonucleotide pools is not complete but is sufficient to inhibit deoxyribonucleic acid (DNA) synthesis, resulting in S-phase cytotoxicity. Arrest of malignant cells in G_1 may result in increased sensitivity to radiation therapy (Bristol-Myers-Squibb, 2005b). The cytotoxic effects are believed to be responsible for the utility of hydroxyurea in treating myeloproliferative disorders such as chronic myeloid leukemia and polycythemia vera (Gwilt and Tracewell, 1998).

The concept of using hydroxyurea in the treatment of sickle cell disease was based initially on the observation that cytotoxic agents increase the production of fetal hemoglobin (hemoglobin F) in non-human primates. The significance of hemoglobin F can be understood in light of the pathophysiology of sickle cell disease. The

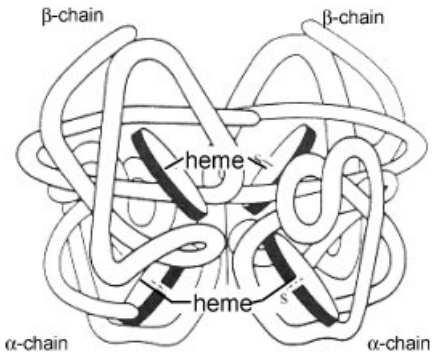

Fig. 2. Hemoglobin structure.

following information was obtained from reviews (Charache et al., 1996; Bunn, 1997; Yang and Pace, 2001; Fixler and Styles, 2002).

Normal hemoglobin is a tetramer of four globin chains, including two α chains and two β chains (Fig. 2). Sickle cell anemia is a hemoglobinopathy in which there is a single A→T mutation in the β-globin gene, resulting in substitution of valine for glutamine in position 6 of the globin chain. In individuals homozygous for the mutant β globin gene, only abnormal β-chains (written β^S) are available for construction of hemoglobin. The abnormal hemoglobin, designated hemoglobin S, polymerizes within the erythrocyte when oxygen tension decreases or local hemoglobin concentration increases. The polymerization of the hemoglobin results in loss of flexibility of the erythrocyte and in abnormal shapes, including crescent- or sickle-shapes. These erythrocytes do not pass normally through the microcirculation, resulting in obstructed blood flow and ischemia in affected tissues. Episodes called vaso-occlusive crises occur when there is a marked increase in sickling, which often occurs in response to a trigger such as infection. During crises, infarction of tissues results in pain and altered organ function. Spleen, lung, kidney, heart, and brain are particularly affected. People with sickle cell anemia show increased rates of destruction of erythrocytes in the microcirculation, due to inflexibility of the erythrocytes, which gives rise to a hemolytic anemia. Sickle cell *disease* is a more general term that includes sickle cell anemia, SC disease[2], and sickle thalassemia. SC disease occurs in people with one A→T mutation (producing a β^S chain) and one A→G mutation (producing a β^C chain). Homozygotes for hemoglobin C (2 α chains and 2 β^C chains) have CC disease. People with CC disease do not get crises. Their only manifestations of disease are typically splenomegaly and a mild anemia. Sickle thalassemia occurs when one β-chain gene bears the S mutation and the other bears mutations that result in reduced or absent transcription of the β-chain. People with β^+ thalassemia make some hemoglobin A and usually have a less severe form of sickle cell disease. People with sickle β^0 thalassemia make no hemoglobin A, and therefore have a clinical course similar to people with SS disease.

[2]SC is not an abbreviation for sickle cell but refers to heterozygosity for hemoglobin S and hemoglobin C.

Sickle cell disease occurs in about 1 in 600 African-Americans (Fixler and Styles, 2002). **[The Expert Panel notes that data on prevalence are not very reliable.]** Other ethnic groups with increased sickle cell disease risk relative to people of western European descent include Greeks, Sicilians, Turks, Arabs, southern Iranians, and Asian Indians. The severity of sickle cell disease is decreased in individuals with elevated production of fetal hemoglobin (hemoglobin F). Hemoglobin F is composed of two α-chains and two γ-chains and is the main hemoglobin produced by the fetus in the second half of pregnancy. All adults produce small amounts of hemoglobin F, typically <1% of their total hemoglobin complement. In some people with sickle cell disease, particularly those who are not of African ancestry, larger than usual amounts of hemoglobin F are produced. Hemoglobin F inhibits the polymerization of hemoglobin S, resulting in milder clinical manifestations of sickle cell disease.

The mechanism by which hydroxyurea increases hemoglobin F production is incompletely understood. It has been proposed that hydroxyurea produces a transient arrest in erythropoiesis followed by a recovery period, during which more immature progenitors that have not yet lost their ability to synthesize hemoglobin F are recruited (Yang and Pace, 2001; Halsey and Roberts, 2003).

Other mechanisms by which hydroxyurea therapy may decrease the incidence and severity of vaso-occlusive crises include reduced expression of adhesion molecules on sickle erythrocytes, improved rheologic properties of erythrocytes through increased hydration of these cells, increased erythrocyte size resulting in lower erythrocyte density, reduced neutrophil number with consequent decreases in pro-inflammatory mediators and in blood viscosity, increased erythropoietin, and increased nitric oxide production resulting in vasodilatation and reduced platelet aggregation (Davies and Gilmore, 2003; Halsey and Roberts, 2003).

The efficacy of hydroxyurea in reducing painful crises in adults was established by the Multicenter Study of Hydroxyurea in Sickle Cell Anemia, a randomized, double-blind, placebo-controlled trial in 299 adults (Charache et al., 1995). There was considerable variability in clinical response and in hemoglobin F levels (Steinberg et al., 1997, 2003).

2.2 Pharmacokinetics and Metabolism

2.2.1 Human

2.2.1.1 Absorption: Hydroxyurea is well absorbed after oral administration. Peak plasma levels occur 1–4 hr after ingestion with water (Bristol-Myers-Squibb, 2002). There are no data on the effect of food on hydroxyurea absorption. Figure 3 shows the mean plasma concentration profile after oral administration of 2000 mg of one brand of hydroxyurea to healthy men. In six adults with sickle cell disease, mean (range) C_{max} after a single oral hydroxyurea dose of 25 mg/kg was 43 (21–54) mg/L, and mean (range) $AUC_{0\rightarrow\infty}$ was 1449 (813–2820) mg-hr/L (Charache et al., 1987). **[The units for area under the curve (AUC) were not given in the study, but were assumed to be 10^{-5} M-hr, consistent with the units used for C_{max}. CERHR converted units to mg-hr/L based on that assumption.]** With larger doses (unspecified),

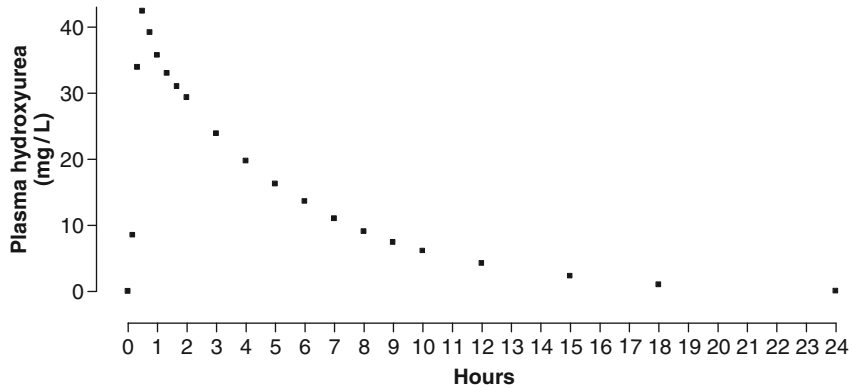

Fig. 3. Mean plasma hydroxyurea in healthy men given hydroxyurea. Hydroxyurea 2000 mg was given by mouth with water at Time 0. From FDA (1998).

Table 3
Plasma Pharmacokinetic Values in Men After Hydroxyurea 2000 mg Orally

Study	C_{max}(mg/L)	T_{max} (hr)	k_{el} (hr^{-1})	$T_{1/2}$ (hr)	$AUC_{0\rightarrow\infty}$ (mg-hr/L)
1-Duramed	50.45	0.91	0.21	3.38	215.39
1-Bristol Myers	48.05	0.84	0.20	3.43	212.96
2-Barr	50.35	0.639	0.196	3.56	217.0
2-Bristol Myers	51.9	0.66	0.198	3.53	218.0

Data taken from applications for generic equivalency by Duramed (FDA, 1998a) and Barr (FDA, 1998b), based on comparison to the Bristol Myers branded product. Studies were carried out in healthy men.
C_{max}, maximum plasma concentration; T_{max}, time to C_{max}; k_{el}, elimination constant; $T_{1/2}$, half-life; $AUC_{0\rightarrow\infty}$, area under the curve from 0 to infinite time.

Appendix II

disproportionately larger increases in peak plasma levels and AUC are seen (Bristol-Myers-Squibb, 2002). According to a review by Stevens (1999), oral absorption is complete; however, a review by Gwilt and Tracewell (1998) indicated 79% oral absorption in patients with cancer.

Pharmacokinetic endpoints are summarized in Table 3. The data were collected in men; no studies were located on women or children. **[The Expert Panel noted that there are good pharmacokinetic data for hydroxyurea in normal men. However, the data in patients are not on par with the data in normal men.]**

2.2.1.2 Distribution: According to the product label and a review study, hydroxyurea distributes in a volume similar to that of total body water and is concentrated in erythrocytes and leukocytes (Stevens, 1999; Bristol-Myers-Squibb, 2002). Hydroxyurea enters the cerebrospinal fluid with peak levels occurring 3 hr after an oral dose (Stevens, 1999). Hydroxyurea was estimated to be 75–80% bound to serum proteins by some authors but was found not to bind to proteins in vitro in human serum (Gwilt and Tracewell, 1998). **[The Expert Panel noted that there are inadequate data to determine binding in serum.]** Continuous intravenous (i.v.) infusion of hydroxyurea 1.0, 2.0, and 3.2 g/m^2/day for 120 hr resulted in steady-state plasma concentrations of 93, 230, and 302 μM **[7, 17, and 23 mg/L]**, respectively (Gwilt and Tracewell, 1998). Another study reported peak plasma concentrations during 72-hour i.v. infusions of hydroxyurea in adults with cancer and data from that study are shown in Figure 4 (Belt et al., 1980). **[The Expert Panel noted that data presented in Figure 4 appear to indicate a linear increase at infusion rates of 2–2.25 mg/m^2/min, but unpredictable increases were observed at infusion rates $\geq$2.5 mg/m^2/min.]**

Milk concentrations of hydroxyurea were measured in a patient who received 1500 mg/day in three divided doses and was sampled 2 hr after the last dose each day for 1 week (Sylvester et al., 1987). The woman had weaned her child during the first 3 days after birth. Only three of the milk samples gave hydroxyurea measurements that were considered interpretable: Day 1 = 6.1 mg/L; Day 3 = 3.8 mg/L; and Day 4 = 8.4 mg/L. Reliable spectrophotographic readings could not be obtained on three of the other samples, because the samples did not clear sufficiently after extraction. The Day 7 sample was not available because of a handling error. **[The blood hydroxyurea concentration in the woman was reported to have been measured on the last day, but was not reported in the study.]** The authors estimated that a nursing infant would receive 3–4 mg/day by this route. **[The Expert Panel noted that depending on the age of the infant, the amount of breast milk ingested could range from 0.3–0.8 L/day, which would provide doses of ~1–6 mg/day under steady-state conditions. Because its plasma half-life is short, ($\leq$1 hr in males, possibly a few hours more in lactating mothers) and hydroxyurea is very water soluble (i.e., low lipid solubility), the dose to the infant would be very dependent on the nursing schedule relative to the mother's ingestion of the drug.]**

2.2.1.3 Metabolism: The metabolic fate of hydroxyurea in humans has not been determined. Metabolism to acetohydroxamic acid by way of a hydroxylamine intermediate has been proposed, but not verified, to account for 1–10% of an oral hydroxyurea dose (Gwilt

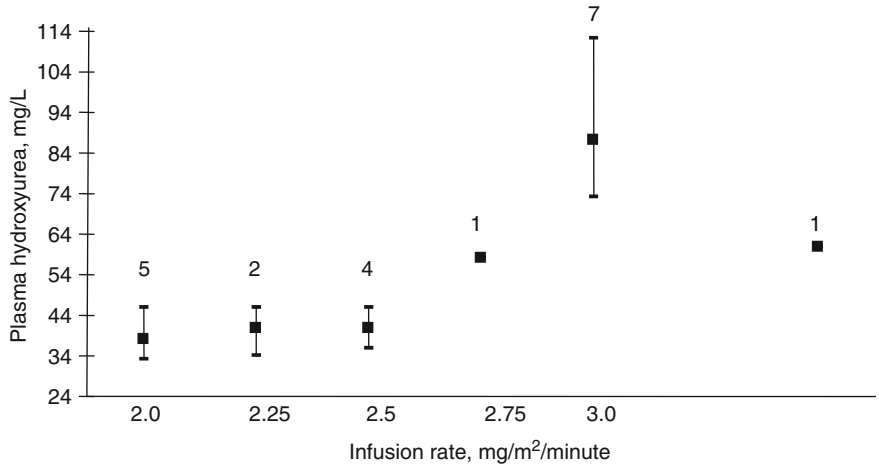

Fig. 4. Plasma hydroxyurea concentration during i.v. infusion of cancer patients. Mean and ranges are illustrated. The numbers above the bars are the number of patients contributing data at each infusion rate. Drawn from data presented by Belt et al. (1980). Molar concentrations were converted for ease of comparison.

and Tracewell, 1998). Dalton et al. (2005) stated that half an administered dose of hydroxyurea is excreted unchanged in the urine and half is metabolized by the liver to carbon dioxide and urea. **[No reference was provided for this statement. The Expert Panel concluded that reliable data on human metabolism of hydroxyurea are not available. There is no information on urinary metabolites.]**

2.2.1.4 Excretion: The product label describes hydroxyurea excretion as nonlinear and as occurring through two pathways (Bristol-Myers-Squibb, 2002). One pathway is saturable and is believed to represent hepatic metabolism, and one pathway is first-order renal excretion. The plasma half-life and elimination constant from studies in normal men given hydroxyurea by mouth are summarized in Table 3. Plasma half-life after stopping an i.v. infusion of hydroxyurea was 220–267 min **[3.67–4.45 hr]** (Belt et al., 1980). Stevens (1999) described elimination of hydroxyurea as biphasic, with a $T_{<FRAX;1;2>\alpha}$ of 1.78 hr and a $T_{<FRAX;1;2>\beta}$ of 3.32 hr. Renal excretion of unchanged hydroxyurea accounts for 36.2% of the oral dose. Renal clearance of hydroxyurea is about 75% of the glomerular filtration rate, or about 5.4 L/hr (Gwilt and Tracewell, 1998). In children with sickle cell disease, hydroxyurea peaked in the urine 2–4 hr after an oral dose and was undetectable 12–15 hr after the dose (Dalton et al., 2005). As indicated in Section 1.2.3, a reduction in dose is recommended for patients with impaired renal function (Yan et al., 2005).

2.2.2 Experimental animal. Animal toxicokinetic studies are summarized below. Studies showed that hydroxyurea is absorbed and distributed throughout the body after oral or intraperitoneal (i.p.) dosing. In pregnant rats, monkeys, and rabbits, hydroxyurea or its metabolites are distributed to the conceptus. Urea is the main hydroxyurea metabolite identified in mouse urine. **[It is not known whether urea is a urinary metabolite of hydroxyurea in humans.]** Urinary excretion is the major elimination route for hydroxyurea, and approximately equal amounts of hydroxyurea and urea are detected in urine after i.p. exposure of mice to hydroxyurea. Elimination of hydroxyurea is rapid, with half-lives reported at $\leq$0.5 hr in rats and mice and $\sim$2 hr in monkeys. Studies examining elimination half-lives in rat or monkey embryos showed slower elimination in the embryo compared to the mother; elimination half-lives in embryos were at least twice those observed in their mothers.

2.2.2.1 Absorption: Detection of ^{14}C-hydroxyurea in the urine of mice and rats after oral dosing indicated that the compound is absorbed through the oral route, as described in more detail in Section 2.2.2.4.

In a study in which 40 pregnant mice were i.p. injected with 300 mg/kg bw hydroxyurea and blood hydroxyurea levels were measured using a colorimetric method for up to 60 min after exposure, a peak hydroxyurea concentration of 311 $\pm$ 22 mg/L **[variance most likely SD]** was measured at 7 min after injection (Warner et al., 1983).

In 5 BALB/c nu/nu (nude) mice/group i.p. injected with hydroxyurea doses ranging from 50–200 mg/kg bw, an absorbance method was used to measure plasma hydroxyurea concentrations at 18–76 min post-exposure, and the study authors concluded that hydroxyurea concentrations were linear ($r^2 = 0.99$) within the dose range administered (Van den Berg et al., 1994). Plasma concentrations of hydroxyurea were $\sim$20 μM **[1.5 mg/L]** after dosing with 50 mg/kg bw, $\sim$150 μM **[11.4 mg/L]** after dosing with 100 mg/kg bw, and $\sim$540 μM **[41 mg/L]** after dosing with 200 mg/kg bw. **[The Expert Panel disagreed with the study authors' conclusion that linear increases in plasma hydroxyurea were observed in the dose range of 50–200 mg/kg. It was noted that for a doubling of the dose (50–100 mg/kg bw), the plasma levels rose 7.5 times and for a 4-fold increase of the dose (50–200 mg/kg bw), the plasma concentration increased 27 times. The kinetics appeared to result from a transition from flow-limited metabolism to capacity-limited metabolism, assuming that urinary excretion remained first order.]** In 6 mice/group that were i.p. injected with 100 mg/kg bw hydroxyurea and killed between 5 and 120 min after exposure, a maximum plasma level of 1465 μM **[111 mg/L]** was observed 10 min after dosing. The AUC was reported at 312.80 μM-min **[24 mg-min/L]**.

2.2.2.2 Distribution: Adamson et al. (1965) examined distribution of ^{14}C-hydroxyurea (99.8% purity) in male

CDBA mice and Fischer rats. Dose levels of hydroxyurea were 100–500 mg/kg bw. Radioactivity levels were measured by liquid scintillation. At 0.5 hr after i.p. dosing of two mice with 500 mg/kg bw ^{14}C-hydroxyurea, the highest levels of radioactivity were measured in carcass (51.6–56.6%), bladder with contents (3.9–10.9%), liver (4.9–5.4%), kidneys (2.8–3.0%), and intestine with contents (3.6%). Lower levels of radioactivity (<1%) were measured in stomach with contents, spleen, heart, and lungs. Recovery of radioactivity was 74–77%. According to the study authors, high levels of radioactivity in kidneys and bladder resulted from rapid excretion occurring predominantly through urine. Failure to recover ~25% of the radioactivity was believed to have resulted from exchange of ^{14}C-carbon dioxide with atmospheric carbon dioxide.

At 1 hr after i.p. dosing of three rats with 100 mg/kg bw ^{14}C-hydroxyurea, the highest concentrations of radioactivity were detected in liver (0.95–2.11%), kidney (1.35–1.75%), and intestine (1.42–2.64%) (Adamson et al., 1965). Lower activities (≤0.55%) were detected in lungs and spleen. **[Total recovery of radioactivity was not reported.]**

In nude mice that were i.p. injected with 200 mg/kg bw hydroxyurea and examined at 18–76 min post-exposure, the highest concentration of hydroxyurea was measured in kidney (~350 μM), followed by lung (113 μM) and brain (199 μM) (Van den Berg et al., 1994). Concentrations in liver were reported to be below the detection limit (≤10 μM). **[Units for tissue concentrations were given as μM. It is not known if units were actually μmol/g, and conversions to mg were therefore not conducted.]** Distribution of hydroxyurea to brain tissue of two to four mice/group was reported to be linear ($r^2 = 0.962$) at i.p. doses of 50–200 mg/kg bw.

Distribution of hydroxyurea was also examined in pregnant animals.

Wilson et al. (1975) compared distribution of hydroxyurea in pregnant rats and monkeys. Embryotoxicity was examined in both species and is discussed in Section 3.2. In both rats and monkeys, embryos and body fluids were examined for hydroxyurea concentration using a colorimetric method. Data were analyzed by Student's *t*-test.

Rhesus monkeys **[gestation period 165 days]** were injected i.v. with 100 mg/kg bw/day hydroxyurea in aqueous solution on gestation day (GD) 23–32, 27–36, or 31–40. The day of vaginal sperm detection was considered GD 0; study authors noted that this method of determining gestational age may have resulted in estimates being off by 24 hr. Blood was collected at 1, 2, 4, 8, 12, or 24 hr after treatment on a day around the mid-point of the treatment period and between the last treatment and when hysterotomies were conducted at 4, 8, or 12 hr after the last injection. At the time of hysterotomy, fluid was drawn from the chorionic and amniotic cavities. Generally, four to nine monkeys/group were examined at each time period before 12 hr post-exposure and one to three monkeys were examined at 12 hr after exposure. Blood levels in monkeys were similar after treatment on GD 23–32, 27–36, and 31–40. Mean maximum concentrations were obtained at 1 hr after dosing at levels ranging from ~76–92 μg/mL. Maximum hydroxyurea blood levels were significantly different at the mid-point compared to the end of the treatment period only after treatment on GD 23–32 (79 compared to 92 mg/L at the mid- vs. end-point of dosing). Authors stated that the results indicated no accumulation or changes in elimination over time or in the period of early compared to late organogenesis. Table 4 compares hydroxyurea levels in maternal and embryonic fluids or tissues. The study authors noted no apparent relationships between hydroxyurea concentrations in maternal and fetal compartments. Hydroxyurea concentration in embryos compared to maternal blood was lower at 4 hr after exposure and higher at 8 and 12 hr after exposure. After treatment on GD 23–32, the half-life of hydroxyurea was estimated at 120 min in maternal plasma and 265 min in embryos. Study authors noted trends for decreased hydroxyurea concentration with increased embryo age **[apparently not tested statistically]**.

Table 4
Hydroxyurea Levels in Maternal and Embryonic Fluids or Tissues After Intravenous Exposure of Pregnant Monkeys to Hydroxyurea on GD 23–32, 27–36, or 31–40

Treatment period (GD)	Time after treatment (hr)	Mean hydroxyurea level (mg/L or mg/kg tissue)			
		Maternal plasma	Chorionic fluid	Amniotic fluid	Embryos
23–32	4	29	48	20	24
27–36	4	30	37	No data	19
31–40	4	33	35	23	15
23–32	8	7	24	19	15
27–36	8	7	33	16	10
31–40	8	7	No data	16	8
23–32	12	2	22	No data	7
27–36	12	2	14	11	5
31–40	12	1	11	15	5

From Wilson et al. (1975). Pregnant animals were given i.v. hydroxyurea 100 mg/kg body weight/day.
GD, gestational day.

Wistar rats were i.p. injected with hydroxyurea at 100, 137, or 175 mg/kg bw/day on GD 9–12 (GD 0 = day of vaginal sperm). Blood was drawn, and embryos were removed from 3–8 dams/time period at 0.25, 0.5, 1, 2, 4, or 8 hr after the last hydroxyurea treatment. Five embryos from each litter were pooled and weighed. Table 5 summarizes mean concentrations of hydroxyurea in maternal blood and embryos at each dose and time period examined. Concentrations of hydroxyurea in embryos exceeded those in maternal blood at ≥1 hr after treatment with 100 mg/kg bw/day and ≥2 hr after treatment with 137 and 175 mg/kg bw/day. The half-life of hydroxyurea in maternal plasma was estimated at 15 min after exposure to 100 or 137 mg/kg bw/day. In embryos, the half-life of hydroxyurea was estimated at 60 min after a dose of 100 mg/kg bw/day and 85 min after a dose of 137 mg/kg bw/day. The study authors noted that hydroxyurea was removed more rapidly from the rat compared to the monkey and, as a result, exposure duration would be shorter in rat than in monkey fetuses.

Beliles et al. (1991) used data from Wilson et al. (1975) and Scott et al. (1971) to develop a pharmacokinetic

Table 5
Hydroxyurea Levels in Maternal and Fetal Tissues After Intraperitoneal Exposure of Pregnant Rats to Hydroxyurea on GD 9–12

		Mean hydroxyurea levels (mg/L or mg/kg tissue)	
Dose (mg/kg body weight/day, i.p.)	Time after treatment (hr)	Maternal plasma	Embryo
100	0.5	47.3	17.6
	1	15.1	21.3
	2	1.2	10.8
	4	0.4	3.0
	8	0.3	0.6
137	0.25	80.6	16.2
	0.5	46.0	21.8
	1	32.8	30.9
	2	2.2	17.4
	4	0.5	6.0
	8	0.2	1.3
175	2	5.6	26.5
	4	0.6	14.1
	8	0.5	1.4

From Wilson et al. (1975).
GD, gestational day.

Table 6
Hydroxyurea Toxicokinetic Variables for Pregnant Rats, Monkeys, and Humans Used in Modeling

Endpoint	Rat	Monkey	Human
Body weight (kg)	0.3	5	60
Apparent maternal volume of distribution (mL)	220	3500	42,600
Volume of embryo compartment (mL)	0.36	6	72
Clearance rate from apparent maternal distribution (hr^{-1})	2.772	0.3465	0.1742

From Beliles et al. (1991).

model describing distribution of hydroxyurea in maternal and embryonic compartments of rats and rhesus monkeys. A three-compartmental model was developed. The compartments represented maternal apparent volume of distribution, embryonic tissues and fluids, and in rats, a pseudo compartment for i.p. exposure. Hydroxyurea transfer from maternal to embryonic compartment was assumed to involve a simple diffusion process. Clearance and metabolism were assumed to occur only in the dam. Interspecies scaling was based on maternal plasma clearance rate and compartmental sizes as a percent of body weight; those endpoints are summarized in Table 6. Experimental animal values were obtained from Wilson et al. (1975), and human values were obtained from Belt et al. (1980).

Predicted values for hydroxyurea concentrations in maternal blood and embryos of rats and monkeys were in general agreement with the actual values reported by Wilson et al. (1975) and summarized in Tables 4 and 5. Table 7 summarizes modeled embryo AUCs resulting from each maternal dose, incidence of affected embryos, and additional risk of embryotoxicity. Additional risk was defined as the risk at a particular dose minus the background risk. Some of the data used to estimate the relationship between pharmacokinetic values and risk were obtained from Scott et al. (1971) and other data **[the majority]** were obtained from the same authors' laboratory **[apparently unpublished data]**. The study authors concluded that maximum susceptibility in rats occurred with exposure on GD 9. Table 8 compares simulated embryo doses in rats, monkeys, and humans and estimates additional risk for humans. In estimating additional risk, humans were assumed to have the same susceptibility as rats and monkeys. Higher embryonic doses were noted in monkeys than rats. The study authors concluded that an i.v. dose of 10 mg/kg bw to a pregnant woman would result in an embryonic concentration of hydroxyurea that did not produce developmental toxicity in rats. In contrast, a human maternal i.v. dose of 50 mg/kg bw hydroxyurea resulted in an embryonic concentration approaching that affecting all monkey fetuses examined. **[It was not stated what types of toxicity were observed in monkey fetuses, and it is not known if the monkey data were ever published.]**

[The Expert Panel recognized the usefulness of the Beliles et al. (1991) model but disagreed with some of the authors' methods and conclusions. Both data sets used to develop the model clearly show diffusion-limited uptake of hydroxyurea into the embryo and clearance from the embryo. For example, the embryo levels lag maternal blood levels shortly after dosing during the uptake phase and also when maternal blood levels are dropping. The Expert Panel concluded that the model does not adequately fit the data, as is suggested by the study authors. In most cases, the study authors needed to "underpredict" or "overpredict" the maternal blood levels to obtain reasonable predictions of hydroxyurea concentrations in the embryo (Table 2, page 273 of the study). The authors stated an assumption of simple diffusion between the embryo and maternal compartments and displayed an equation at the bottom of page 270. A limitation of the model identified by the Expert Panel (without doing the analysis) is that the transfer rate constant (K_t; 73.73/hr) for hydroxyurea transfer in and out of the embryo is a first-order constant and is multiplied by the calculated *mass* of hydroxyurea in the maternal compartment and embryo. The calculated mass is very large in the maternal compartment and small in the embryo. The preferred method would be to use a permeability-area constant, cross product (PA, L/hr) based on a predicted *concentration* in the embryo and maternal compartment to drive the kinetics. Therefore verification is required before applying the model for risk prediction.]

Distribution of hydroxyurea to rabbit fetuses was observed by a colorimetric method from 15 min to 8 hr after subcutaneous (s.c.) injection of pregnant rabbits with 650 mg/kg bw hydroxyurea (DeSesso and Goeringer, 1990b). Hydroxyurea levels rose steadily over 3 hr, concentrations remained steady at ~2.8–3.2 μg hydroxyurea/mg protein from 3–6 hr after treatment, and then concentrations began to decline 8 hr after treatment.

2.2.2.3 Metabolism: Adamson et al. (1965) examined metabolism of hydroxyurea in CDBA mice i.p. dosed with 500 mg/kg bw ^{14}C-hydroxyurea. Hydroxyurea and its metabolites were identified in urine and exhaled air

Table 7
Maternal Dose and Embryonic Exposure to Hydroxyurea on Each GD and Effects on Embryo Responses and Additional Risk in Rats

Exposure (GD)	Maternal dose (mg/kg body weight)	Embryo AUC (mg-hr/L)	Affected embryos	Additional risk (%)
9	100	6–9	11/115	3.1
	250	181	270/280	89.9
10	300	222	52/185	21.6
	375	284	104/118	81.6
	500	395	110/118	86.7
11	250	181	4/46	2.2
	500	395	41/73	49.7
	650	537	64/64	[a]
12	250	181	7/147	[a]
	500	395	27/154	11.1
	750	639	101/139	66.2
	1,000	912	155/155	[a]
Historic control	0	0	31/481 (6.4%)	—

From Beliles et al. (1991).
[a]Responses of 100% or less than the control background were not used in the calculation of additional risk.
GD, gestational day.

Table 8
Simulated Embryo Doses in Rats on GD 9–12, Monkeys on GD 21–44, and Humans Exposed to Hydroxyurea

Maternal dose (mg/kg body weight/day)	Exposure (GD)	Simulated embryo AUC (mg-hr/L)	No. affected/no. implants (animals) or estimated additional risk (humans)
Rat			
175	9–12	124	42/68
137	9–12	95	42/84
100	9–12	69	8/105
0	9–12		31/481
Monkey			
100	21–44	392	6/6
Human			
50	Unknown	353	10–100%
10	Unknown	69	0–3.1%

From Beliles et al. (1991).
GD, gestational days.

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by high-voltage paper electrophoresis. In urine collected from metabolic cages or directly from the bladder between 3–24 hr after dosing, approximately equal amounts of ^{14}C-hydroxyurea (27–44%) and ^{14}C-urea (31–42%) were detected. Smaller amounts of radioactivity ($\leq$7%) were present as ^{14}C-carbon dioxide in expired air and ^{14}C-carbonate in urine. Similar metabolism patterns were observed in a mouse that was pretreated with 500 mg/kg bw/day non-radioactive hydroxyurea for 6 days before it was dosed with radiolabeled hydroxyurea at 500 mg/kg bw and in germ-free mice that were i.p. dosed with 500 mg/kg bw ^{14}C-hydroxyurea.

Adamson et al. (1965) also examined in vitro conversion of ^{14}C-hydroxyurea to ^{14}C-urea in minced or homogenated mouse tissues. Metabolic conversion was highest in liver and kidney and very low in lung, spleen, and small intestine.

2.2.2.4 Elimination: Adamson et al. (1965) examined excretion of ^{14}C-hydroxyurea in male CDBA mice and Fischer rats using a liquid scintillation counting technique. ^{14}C-Hydroxyurea treatment regimens were 500 mg/kg bw by i.p. injection in four studies, 200 mg/kg bw by i.p. injection in one study, and 200 mg/kg bw orally in one study. **[The number of mice treated in each study was not reported, and it is possible that each study used only one mouse. The oral route was not further specified. If only one mouse was used, the study is not of high utility.]** Urinary excretion was the major route of elimination with 82–91% of the dose eliminated within 24 hr of exposure to either dose through either route. After administration of 500 mg/kg bw by i.p. injection, 64–75% of the dose was eliminated in urine within 3 hr. Urinary elimination was similar with oral and i.p. dosing at 200 mg/kg bw. Percent radioactive dose in urine with i.p./oral dosing was 76/63% at 4 hr, 86/79% at 8 hr, and 90/91% at 24 hr after dosing. In mice receiving an i.p. injection of 500 mg/kg bw hydroxyurea, 7% of the dose was detected in exhaled air within 24 hr of exposure. No more than 0.5% of the dose was present in feces within 24 hr after i.p. dosing with 200 or 500 mg/kg bw or oral dosing with 200 mg/kg bw. In those studies, 64–95% of the dose was recovered. Similar patterns of urinary elimination were observed after s.c. pretreatment of one mouse with non-radioactive

hydroxyurea (500 mg/kg bw/day for 6 days) before it was i.p. dosed with radiolabeled hydroxyurea at 500 mg/kg bw and in germ-free BALB/c mice i.p. injected with radiolabeled hydroxyurea at 500 mg/kg bw/day.

The doses examined in rats were 100 mg/kg bw administered by i.p. injection and 50 mg/kg bw administered orally. **[The number of rats treated in each study was not reported, and it is possible that each study used one rat. The oral route was not further specified. If only one rat was used, the study is not of high utility.]** Urinary excretion was the major route of elimination of hydroxyurea. At 24 hr after i.p. dosing, 90% of radiolabel was detected in urine. At 24 hr after oral exposure, 57% of radiolabel was detected in urine and 13.8% was detected in expired air. Radioactive activity detected in feces was 0.3–0.8% at 24 hr after exposure through either route. Recovery of radiolabel was 90% with i.p. dosing and 72% with oral dosing.

A terminal half-life of 11.3 min for hydroxyurea clearance from plasma was reported in six nude mice/group i.p. injected with 100 mg/kg bw hydroxyurea and examined for up to 120 min after exposure (Van den Berg et al., 1994). A number of studies reported elimination half-lives in pregnant animals and in some cases, in their unborn offspring. Half-life in maternal plasma was estimated at 30 min in 40 pregnant mice that were i.p. injected with 300 mg/kg bw hydroxyurea on GD 9 (Warner et al., 1983). Elimination half-lives were reported at 20 min in maternal rat blood and 45 min in fetuses after i.p. dosing of the dam with 250 mg/kg bw hydroxyurea (Rajewsky et al., 1971). Half-life of hydroxyurea in maternal plasma was estimated at 15 min after exposure of rats to hydroxyurea at 100 or 137 mg/kg bw/day (Wilson et al., 1975); in embryos of those rats, estimated half-lives were 60 min at a dose of 100 mg/kg bw/day and 85 min at a dose of 137 mg/kg bw/day. In monkeys i.v. dosed with 100 mg/kg bw/day hydroxyurea on GD 23–32, half-lives were estimated at 120 min in maternal plasma and 265 min in embryos (Wilson et al., 1975). **[The Expert Panel concluded that hydroxyurea is present for a longer time period in the bodies of embryos than in their mothers.]**

Table 9
Non-Hematologic Adverse Reactions to Hydroxyurea Listed in the Product Label

Adverse Reaction
Gastrointestinal symptoms
Stomatitis
Anorexia
Nausea
Vomiting
Diarrhea
Constipation
Dermatologic
Maculopapular rash
Skin ulceration
Dermatomyositis-like skin changes
Peripheral and facial edema
Hyperpigmentation
Atrophy of skin and nails
Scaling
Violet papules
Skin cancer
Dysuria
Alopecia
Drowsiness
Neurological disturbances (“extremely rare”)
Headache
Dizziness
Disorientation
Hallucinations
Convulsions
Temporary impairment of renal tubular function
Fever
Chills
Malaise
Edema
Asthenia
Elevation of hepatic enzymes

From the product label (Bristol-Myers-Squibb, 2004).

2.3 General Toxicology

2.3.1 Human. According to the product label, the principal toxicity of hydroxyurea is hematologic, with suppression of bone marrow (Bristol-Myers-Squibb, 2004). Neutropenia is the most common hematologic adverse effect, although thrombocytopenia and anemia can occur. Secondary leukemias have been reported in patients who received hydroxyurea for myeloproliferative disorders (Discussion in Section 2.5, below). Less frequent adverse effects listed in the label are shown in Table 9.

Although skin ulceration is seen in patients treated with hydroxyurea for myeloproliferative disorders, a report on 17 adults with sickle cell disease who were treated with hydroxyurea found that five (29%) developed leg ulcers (Chaine et al., 2001). The authors suggested that the underlying disease, younger age, longer treatment periods, or darker skin types of sickle cell patients might be responsible for increased susceptibility to this adverse effect of hydroxyurea. **[When skin ulceration occurs in patients with sickle cell disease, it is unclear whether the ulceration is related to the underlying disease or the hydroxyurea.]**

2.3.2 Experimental animal. Drug labels reported oral LD_{50}s for hydroxyurea at 7330 mg/kg bw in mice and 5780 mg/kg bw in rats (FDA, 1998a; Bristol-Myers-Squibb, 1999, 2002, 2004, 2005b). Table 10 lists hydroxyurea LD_{50}s reported in the ChemIDplus database (ChemIDplus, 2004).

Most of the information on general toxicity effects in animals after repeated dosing with hydroxyurea was found in drug labels and was very limited (FDA, 1998a; Bristol-Myers-Squibb, 1999, 2002, 2004, 2005b). **[As noted in the summary of the information presented below, species and effective doses were not always clearly specified. Therefore, the information is of limited use but can provide some qualitative information on the types of toxic effects that can occur.]** In some laboratory animal species given doses that exceeded clinical levels, observations included cardiovascular effects (e.g., changes in heart rate, blood pressure, and electrocardiogram [EKG] and development of orthostatic hypotension) and hematologic effects (slight hemolysis and methemoglobinemia). In subacute and chronic toxicity studies in rats, an apparently dose-related and

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Table 10
LD_{50} Values for Hydroxyurea

Species	Exposure route	LD_{50} (mg/kg body weight)
Dog	Intravenous	>1000
	Oral	>2000
Mouse	Intraperitoneal	5800
	Intravenous	2350
	Oral	7330
Rat	Intraperitoneal	>4700
	Intravenous	4730
	Oral	5760

From ChemIDplus (2004).

mild-to-moderate bone marrow hypoplasia was observed, in addition to pulmonary congestion and mottled lungs. Testicular atrophy and lack of spermatogenesis were observed after a 37-day exposure to 1260 mg/kg bw/day and a 40-day exposure to 2520 mg/kg bw/day hydroxyurea. Hepatic damage with fatty meta morphogenesis also occurred in rats exposed to hydroxyurea. Effects observed in dog studies included mild-to-marked bone marrow depression **[apparently at >140 mg/kg bw/day]**. At higher dose levels (140–420 or 140–1260 mg/kg bw/week given 3 or 7 days/week for 12 weeks), growth retardation, slightly increased blood glucose levels, hemosiderosis of liver or spleen, and reversible spermatogenic arrest were observed. Effects in monkeys exposed to hydroxyurea included bone marrow depression, lymphoid atrophy of spleen, and degenerative changes in epithelium of small and large intestine **[testes not mentioned]**. At higher and often lethal doses (400–800 mg/kg bw/day administered for 7–15 days), hemorrhage and congestion were observed in lungs, brain, and urinary tract. A review by Gwilt and Tracewell (1998) reported that a study conducted in 1928 observed leukopenia, macrocythemia, anemia, and death in animals exposed to hydroxyurea.

2.4 Genetic Toxicity

2.4.1 Human. Charache et al. (1987) described an increase in chromosome breaks in peripheral blood mononuclear cells in four of six sickle cell disease patients on hydroxyurea compared to untreated sickle cell patients; however, in two of the patients with pre-hydroxyurea evaluations, no increase in chromosome breaks on hydroxyurea had occurred. One hydroxyurea-treated sickle cell disease patient also had an elevation in rearrangements, as did one untreated sickle cell disease patient. **[No information was given on statistical methods. In addition, hydroxyurea-treated patients had received other treatments. Therefore, the utility of the data is questionable.]** Loukopoulos et al. (2000) reported no chromosome aberrations and no increase in sister chromatid exchange in peripheral lymphocytes of 10 patients receiving hydroxyurea "over several years" for sickle cell disease/β-thalassemia compared to matched controls. **[Few methodologic details were given, and no data were shown. Therefore, the study is not useful.]** Khayat et al. (2006) reported no increase in lymphocyte chromosome anomalies in eight patients aged 7–20 years with sickle cell disease who were monitored before hydroxyurea therapy and then every 2 months while on therapy for 1 year. **[The Expert Panel concluded that this study is useful.]**

Weinfeld et al. (1994) carried out repeated cytogenetic examinations on patients treated with hydroxyurea for myeloproliferative disorders. Among 19 patients untreated previously who had an initial normal karyotype, seven (37%) developed clonal abnormalities. Three of six patients treated previously with normal karyotypes at the start of hydroxyurea treatment developed chromosomal abnormalities. The chromosomes affected most commonly were 1, 9, 12, and 13. **[The Expert Panel concluded that this study is useful.]**

Hanft et al. (2000) evaluated acquired mutations in 27 adults with myeloproliferative disorder (15 of whom had 0–21 months of hydroxyurea exposure and 12 of whom had 4–18 years of hydroxyurea exposure) and 30 adults with sickle cell disease (15 of whom were exposed to hydroxyurea for a median of 24 months and 15 of whom were unexposed age-matched controls). **[Children were also evaluated and are discussed in Section 3.1.3.2.]** Mononuclear cells were isolated from peripheral venous blood and used to detect mutations at the hypoxanthine phosphoribosyl transferase (*HPRT*) locus. T cell receptor interlocus recombination events (at the Vγ and Jβ loci) were also evaluated. Hydroxyurea therapy was not associated with a statistically significant increase in *HPRT* mutant frequency or Vγ-Jβ recombination events. **[These studies are limited by lack of control data, small sample, and insufficient length of follow-up.]**

2.4.2 Experimental animal. Assessment of mutagenicity associated with hydroxyurea was based primarily on an IARC review (IARC, 2000). A limited number of studies that were not included in the IARC review were summarized by CERHR. Results of in vitro genetic toxicity testing are included in Table 11, and results of in vivo toxicity tests are included in Table 12. IARC concluded that hydroxyurea did not induce mutations in bacteria or at the *HPRT* locus of mammalian cells. Mutations were observed at the *Tk* locus of mouse lymphoma cells. IARC noted that hydroxyurea induced recombination in yeast and sister chromatid exchanges and gene amplification in mammalian cells. Transformation was observed in some but not all cell lines. Clastogenic activity of hydroxyurea was shown in the majority of in vitro and in vivo studies examining that endpoint. IARC concluded that hydroxyurea was ineffective in inducing germ cell mutations but noted that extensive testing was not conducted. **[One study not included in the IARC review reported a moderate increase in mutant frequencies in spermatogonia (Martus et al., 1999).]**

According to information provided in drug labels, hydroxyurea induced mutagenicity in bacteria **[not otherwise specified]**, fungi, protozoa, and mammalian cells and induced clastogenic responses in hamster cells and human lymphoblasts in vitro (Bristol-Myers-Squibb, 2001a,b, 2002, 2004, 2005a,b). In vivo studies showed induction of sister chromatid exchanges in rodents and micronuclei in mice after hydroxyurea exposure. Hydroxyurea transformed rodent embryo cells to a tumorigenic phenotype. Hydroxyurea was classified as an unequivocal genotoxicant in drug labels (Bristol-Myers-Squibb, 2005a).

Table 11
In Vitro Genetic Toxicity Studies of Hydroxyurea

Concentration	Cell	Endpoint	Results	Reference
0.05, 0.5, 5, 50, and 500 μg/plate; metabolic activation	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100	Mutation	↔	Bruce and Heddle (1979)
10,000 μg/plate with and without metabolic activation	*Salmonella typhimurium* strains TA100, TA1535, TA1537, TA98	Mutation	↔	Haworth et al. (1983)[a]
10,000 μg/mL without metabolic activation	*Saccharomyces cerevisiae* strain D5	Mutation	↔	Ferguson and Turner (1988a)[a]
3 μg/mL without metabolic activation	Mouse lymphoma L5178Y cells, *Tk* locus	Mutation	↑	Amacher and Turner (1987)[a]
0.7 μg/mL without metabolic activation	Mouse lymphoma L5178Y cells, *Tk* locus	Mutation	↑	Wangenheim and Bolcsfoldi (1988)[a]
20 μg/mL with and without metabolic activation	Mouse lymphoma L5178Y cells, *Tk* locus	Mutation	↑	Sofuni et al. (1996)[a]
19 μg/mL without metabolic activation	T-lymphoblast cells, *HPRT*, locus	Mutation	↔	Mattano et al. (1990)[a]
7600 μg/mL without metabolic activation	*Escherichia coli* K12	SOS repair	↑	Barbé et al. (1987)[a]
760 μg/mL without metabolic activation	Rat primary hepatocytes	Unsheduled DNA synthesis	↑	Rossberger and Andrae (1985)[a]
38 μg/mL without metabolic activation	Ehrlich ascites tumor cells	DNA single strand breaks	↑	Li and Kaminskas (1987)[a]
4.6 μg/mL without metabolic activation	Human T lymphoma CCRF-CEM cells	DNA single strand breaks	↑	Skog et al. (1992)[a]
2400 μg/mL without metabolic activation	*Saccharomyces cerevisiae* strain D5	Mitotic crossing over	↑	Ferguson and Turner (1988b)[a]
7600 μg/mL without metabolic activation	*Saccharomyces cerevisiae* strain D61.M	Mitotic gene conversion	↑	Mayer et al. (1986)[a]
0.5 μg/mL without metabolic activation	Chinese hamster lung V79-4 cells	Sister chromatid exchanges	↔	Popescu et al. (1977)[a]
7.6 μg/mL without metabolic activation	Chinese hamster lung V79 B-1 cells	Sister chromatid exchanges	↑	Ishii and Bender (1980)[a]
	Chinese hamster lung V79 and ovary cells	Sister chromatid exchanges	↑	Mehnert et al. (1984)[a]
23 μg/mL without metabolic activation	Chinese hamster ovary CHO-B11 cells	Sister chromatid exchanges	↑	Hahn et al. (1986)[a]
76 μg/mL without metabolic activation	Mouse lymphoma L5178Y Jsens and C3 cells	Sister chromatid exchanges	↑	Hill and Schimke (1985)[a]
76 μg/mL without metabolic activation	Chinese hamster ovary CHO-K1 cells	Sister chromatid exchanges	↑	Tohda and Oikawa (1990)[a]
7600 μg/mL without metabolic activation	*Saccharomyces cerevisiae* strain D61.M	Aneuploidy	↑	Mayer et al. (1986)[a]
380 μg/mL without metabolic activation	*Saccharomyces cerevisiae* strain RS112	Intrachromosomal recombination	↑	Galli and Schiestl (1996)[a]
2280 μg/mL without metabolic activation	*Saccharomyces cerevisiae* strains SBTD and D7	Ultraviolet-induced mitotic gene conversion	↑	Zaborowska et al. (1983)[a]
3040 μg/mL without metabolic activation	*Saccharomyces cerevisiae* strains 419 and 580	Meiotic recombination	↑	Simchen et al. (1976)[a]
1520 μg/mL without metabolic activation	Human primary lymphocytes	Micronuclei	↔	Fenech et al. (1994)[a]
10–18 μg/mL without metabolic activation	Mouse L5178Y cells	Micronuclei	↑ at ≥10 μg/mL	Avlasevich et al. (2006)
0.5, 1.0, or 10.0 μg/mL; activation unknown	Rat embryonic cell tissue	Metaphase figures with chromosomal aberrations	Aberration rates 0% at 0.5 μg/mL, 14% at 1.0 μg/mL, 7% at 10 μg/mL; in 6 of 9 experiments, only mitotic inhibition observed	Soukup et al. (1967)
7.6 μg/mL without metabolic activation	*Drosophila melanogaster* larvae	Chromosomal aberrations in brain ganglion cells	↑	Banga et al. (1986)[a]

Table 11
Continued

Concentration	Cell	Endpoint	Results	Reference
150 μg/mL without metabolic activation	Human lymphocytes	Chromosomal aberrations	↑	Kihlman and Anderson (1985)[a]
190 μg/mL without metabolic activation	Human Hep2 cell line	Chromosomal aberrations	↑	Strauss et al. (1972)[a]
76 μg/mL without metabolic activation	Human B lymphoblast TK6, WI-L2-NS and WTK1 cells	Chromosomal aberrations	↑	Greenwood et al. (1998)[a]
0.5 μg/mL without metabolic activation	Chinese hamster lung V79-4 cells	Chromosomal aberrations	↑	Popescu et al. (1977)[a]
23 μg/mL without metabolic activation	Chinese hamster ovary CHO-B11 cells	Chromosomal aberrations	↑	Hahn et al. (1986)[a]
100 μg/mL without metabolic activation	Chinese hamster Don-C cells	Chromosomal aberrations	↑	Karon and Benedict (1972)[a]
76 μg/mL without metabolic activation	Mouse lymphoma L5178Y Jsens and C3 cells	Chromosomal aberrations	↑	Hill and Schimke (1985)[a]
0.76 μg/mL without metabolic activation	Embryonic cells from BN/a mice (confirmation in newborn mice)	Cell transformation	↑	Chlopkiewicz and Koriorowska (1983)[a]
Concentration not reported; no metabolic activation	Embryonic cells from DBA/2 and Swiss mice	Cell transformation	↔	Chlopkiewicz and Koriorowska (1983)[a]
7.6 μg/mL without metabolic activation	BALB/c 3T3 mouse cells	Cell transformation	↔	Chlopkiewicz and Koriorowska (1983)[a]

[a]Cited in IARC (2000), in which concentrations are listed at lowest effective dose or highest ineffective dose.
↑, Genotoxicity response; ↔, no genotoxicity response.

Appendix II

2.5 Carcinogenicity

2.5.1 Human. There have been a number of case reports of acute leukemia and skin cancers in patients who have been treated with hydroxyurea for myeloproliferative disorders (Hanft et al., 2000; IARC, 2000). Weinfeld et al. (1994) followed 50 adults on hydroxyurea for polycythemia vera, essential thrombocythemia, or myeloid metaplasia and noted the development of acute leukemia in nine of them, with a myelodysplastic syndrome[3] developing in another patient. Seven of the patients who developed leukemia were treated with hydroxyurea alone. Hydroxyurea was used for 5–111 months before the diagnosis of acute leukemia. Sterkers et al. (1998) found acute myeloid leukemia or a myelodysplastic syndrome in seven (3.5%) of 201 patients treated with hydroxyurea alone and 14 (5.5%) of 251 patients in whom hydroxyurea was used with or without other agents. About 40% of essential thrombocythemia patients who developed leukemia or a myelodysplastic syndrome on hydroxyurea had a 17p deletion. Chim et al. (2005), citing their experience in Hong Kong and reviewing six other reports, estimated the incidence of leukemia or a myelodysplastic syndrome at 1.3–4.5% after hydroxyurea as the only therapy for essential thrombocythemia. **[The Expert Panel concluded that data from the Weinfeld et al. (1994) and Sterkers et al. (1998) studies are of high utility.]**

Najean and Rain (1997) calculated an actuarial risk of leukemia or myelodysplastic syndrome at ~10% by the 13th year of therapy in patients treated with hydroxyurea for polycythemia vera. The risk of other cancer was calculated as ~15% by the 14th year, or about 1.1% annually, which the authors considered to be only slightly greater than the age-adjusted general population rate of 0.8% annually. The cancers diagnosed in patients on hydroxyurea involved the lung, pleura, skin, thyroid, pancreas, and vagina. **[The Expert Panel concluded that data from this study are useful.]**

IARC (2000) concluded that available data did not allow a conclusion on whether the occurrence of acute leukemia or myelodysplastic syndrome in patients treated with hydroxyurea for myeloproliferative disorders represented progression of the myeloproliferative disorder or an effect of treatment.

Although there have been two case reports of leukemia in children on hydroxyurea for sickle cell disease, the short durations of therapy (7 weeks and 6 months) before leukemia diagnosis makes a causal relationship less likely (Amrolia et al., 2003). In addition, leukemia in adults on hydroxyurea for sickle cell disease has not been reported, but the longest published follow-up is 9 years (Steinberg et al., 2003); longer follow-up is needed. Children treated with hydroxyurea are expected to have longer periods of exposure given their longer life expectancy.

2.5.2 Experimental animal. The drug label states that there are no conventional long-term studies for hydroxyurea, but one study reported tumors in rats exposed to hydroxyurea (Bristol-Myers-Squibb, 2005a). An increased incidence of mammary tumors compared to controls at 18 months was observed in female rats i.p. injected with 125–250 mg/kg bw hydroxyurea (~0.6–1.2 times the maximum recommended human oral daily dose on a mg/m^2 basis) 3 times/week for 6 months.

[3]The myelodysplastic syndromes, which used to be called "preleukemia," are characterized by ineffective production of blood cells and varying risks of transformation to acute myeloid leukemia. Myelodysplastic syndromes are not true malignancies but are usually classed as hematologic neoplasms.

Table 12
In Vivo Genetic Toxicity Studies of Hydroxyurea

Model	Dosage (mg/kg body weight) (route)	Cells	Endpoint	Results	Reference
101/H male × C3H/HeH (female)F_1 mouse	500/day × 2 (i.p.)	Spermatogonia	Specific locus mutation	↔	Cattanach et al. (1989)[a]
ICR mouse dams	0 or 250 on GD 10	Embryo	Lateral asymmetry (unequal banding of sister chromatids)	↔ at 4 hr after exposure	Tucci and Skalko (1979)
"Commercial strain" of rat dams	0, 750, or 1500 on GD 13 (injection)	Embryo	Aberrations in metaphase figures	↔ at 6–24 hr after exposure	Soukup et al. (1967)
Sprague-Dawley rat dams	0, 180, 360, or 720 on GD 13 (i.p.)	Maternal erythrocytes	Micronuclei	↑3-fold at ≥360 mg/kg bw	Awogi et al. (1987) (abstract)
		Fetal erythrocytes	Micronuclei	↑2–3-fold at 180 mg/kg bw and 18-fold at 720 mg/kg bw	
Adult female C57BL/6 × C3H/He mice	~250–2000/day for 5 days (i.p.)	Bone marrow	Micronuclei	↔ at 4 hr after exposure	Bruce and Heddle (1979)
Male NMRI mouse	400 (i.p.)	Bone marrow	Micronuclei	↑	Hart and Hartley-Asp (1983)[a]
Drosophila melanogaster larvae	6080	Brain ganglia	Chromosomal aberrations	↑	Banga et al. (1986)[a]
Male Swiss mice	500 (i.p.)	Spermatogonial cells	Chromosomal aberrations	↔	Van Buul and Bootsma (1994)[a]
Adult male C57BL/6C3H/He mice		Sperm	Abnormalities in morphology **[according to IARC (2000)]**	↑ at ~ ≥250 mg/kg bw/day 35 days after exposure	Bruce and Heddle (1979)
Adult male Swiss mice	250 or 500 (i.p.)	Stem cell spermatogonia	Translocations at 99–105 days after treatment	↔	van Buul and Goudzwaard (1990)
Male ICR/Ha Swiss mice	1000 (i.p.)	Male germ cells	Dominant lethality	↔	Epstein et al. (1972)[a]
Transgenic (pUR 2888 plasmid) C57B1/6J male adult mice	0 × 2 or 500 × 2 (i.p.), 3 hr apart	Spermatogonia	Mutant frequency	↑4-fold at 75 days after exposure	Martus et al. (1999)
		Lung		↑3-fold at 75 days after exposure	
		Spleen		↑1.5-fold at 75 days after exposure	

[a]Cited in IARC (2000), in which concentrations are listed at lowest effective dose or highest ineffective dose.
↑, Genotoxicity response; ↔, no genotoxicity response; i.p., intraperitoneal; GD, gestational day.

IARC (2000) reviewed a study in which 50 XVII/G mice were i.p. injected with hydroxyurea at doses of 1 mg at 2 days of age, 3 mg at 8 days, 5 mg at 15 days, and 10 mg/week from 30 days to 1 year of age. A control group of 50 animals was not treated. The incidence of pulmonary tumors was reported at 46% in the hydroxyurea group, 60% in the negative control group, and 93% in the positive control group treated with urethane. The IARC review also noted a number of studies that evaluated carcinogenic responses in animals treated with hydroxyurea in combination with carcinogens, but concluded that the studies were not adequate for assessing carcinogenicity of hydroxyurea. IARC concluded that "There is *inadequate evidence* in experimental animals for the carcinogenicity of hydroxyurea." In their overall evaluation, IARC concluded, "Hydroxyurea is *not classifiable as to its carcinogenicity to humans (Group 3)*."

2.6 Potential Sensitive Subpopulations

People with myeloproliferative disorders may be susceptible to possible carcinogenic effects of hydroxyurea. No information was located indicating that children and adults differ in sensitivity to hydroxyurea toxicity; however, some authors of studies on use of hydroxyurea in children (discussed in Section 3.1) have commented that children do not appear to be more sensitive to hydroxyurea toxicity.

Table 13
Human Pharmacologic Data for Hydroxyurea

Endpoint	Value	Reference
Oral absorption	≥79%	Gwilt and Tracewell (1998); Stevens (1999)
T_{max}	~0.6–4 hr	Bristol-Myers-Squibb (2002); FDA (1998a,b)
C_{max}		
Sickle cell patients given 25 mg/kg bw	43 (21–54) mg/L	Charache et al. (1987)
Healthy men given ~29 mg/kg bw	48–52 mg/L	FDA (1998a,b)
$AUC_{0\rightarrow\infty}$ (healthy men given ~29 mg/kg bw)	213–218 mg-hour/L	FDA (1998a,b)
Volume of distribution	Similar to total body water; concentrates in erythrocytes and leukocytes	Bristol-Myers-Squibb (2002); Stevens (1999)
Cerebrospinal fluid T_{max}	3 hr	Stevens (1999)
Serum protein binding	75–80% **[The Expert Panel does not consider the protein binding data reliable.]**	Gwilt and Tracewell (1998)
Plasma half-life	~3.4–4.5 hr after oral or intravenous infusion exposures	Belt et al. (1980); FDA (1998a,b)

Table 14
Experimental Animal Pharmacologic Data for Hydroxyurea

Endpoint	Model	Value	Reference
C_{max} after intraperitoneal injection of 300 mg/kg bw	Mouse, pregnant	311 mg/L	Warner et al. (1983)
C_{max} after intraperitoneal injection of 40–200 mg/kg bw	Nude mouse	**[1.5–41 mg/L, not dose proportional]**	Van den Berg et al. (1994)
Metabolic fate	Mouse	Unchanged hydroxyurea + urea in equal amounts in urine within 24 hr	Adamson et al. (1965)
Elimination half-life	Mouse and rat, pregnant or non-pregnant, exposed intraperitoneally	11–30 min	Rajewsky et al. (1971); Van den Berg et al. (1994); Warner et al. 1975, 1983)
Elimination half-life	Monkey exposed intravenously	120 min	Wilson et al. (1975)
Renal excretion	Mouse exposed orally or intraperitoneally	82–91%	Adamson et al. (1965)
	Rats exposed intraperitoneally	90%	Adamson et al. (1965)
	Rats exposed orally	57%	Adamson et al. (1965)

Appendix II

2.7 Summary of General Toxicology and Biological Effects

2.7.1 Pharmacodynamics. Chemotherapeutic uses of hydroxyurea are based on its inhibition of ribonucleotide reductase, which catalyzes the conversion of ribonucleotides to deoxyribonucleotides (Koç et al., 2004). Depletion of deoxyribonucleotide pools lead to inhibition of deoxyribonucleic acid (DNA) synthesis, resulting in S-phase cytotoxicity. Arrest of malignant cells in G_1 may increase sensitivity to radiation therapy (Bristol-Myers-Squibb, 2005b). Cytotoxicity is believed to be responsible for the utility of hydroxyurea in myeloproliferative disorders such as chronic myeloid leukemia and polycythemia vera (Gwilt and Tracewell, 1998).

The concept of using hydroxyurea in the treatment of sickle cell disease was based initially on the observation that cytotoxic agents increase the production of fetal hemoglobin (hemoglobin F) in non-human primates. Hemoglobin F inhibits the polymerization of hemoglobin S, resulting in milder clinical manifestations of sickle cell disease. Other mechanisms by which hydroxyurea therapy may decrease the incidence and severity of vaso-occlusive crises include reduced expression of adhesion molecules on sickle erythrocytes, improved rheologic properties of erythrocytes through increased hydration of these cells, increased erythrocyte size resulting in lower erythrocyte density, reduced neutrophil number with consequent decrease in pro-inflammatory mediators and in blood viscosity, increased erythropoietin, and increased nitric oxide production resulting in vasodilatation and reduced platelet aggregation (Davies and Gilmore, 2003; Halsey and Roberts, 2003).

2.7.2 Pharmacokinetics. Pharmacokinetic data for adult humans are summarized in Table 13.

In a nursing mother who received 1500 mg/day hydroxyurea in three divided doses, milk was sampled 2 hr after the last dose each day for 1 week (Sylvester et al., 1987). Reliable estimates of hydroxyurea were only obtained on three of the days of testing: Day 1 = 6.1 mg/L; Day 3 = 3.8 mg/L; and Day 4 = 8.4 mg/L. **[Based on this single study of one woman, the Expert Panel estimated**

that nursing infants could potentially be exposed to ~1–6 mg/day hydroxyurea under steady state conditions. The infant dose would be very dependent on the nursing schedule relative to the mother's ingestion of the drug and the volume of feeds.]

No reliable information was found for metabolism of hydroxyurea by humans including infants, children, and adolescents. The product label describes hydroxyurea excretion as nonlinear and as occurring through two pathways (Bristol-Myers-Squibb, 2002). A saturable pathway is believed to represent hepatic metabolism, and a second pathway, first-order renal excretion. Renal excretion of unchanged hydroxyurea accounts for ~36% of the oral dose and is ~75% of the glomerular filtration rate (~5.4 L/hr) (Gwilt and Tracewell, 1998). In children with sickle cell disease, hydroxyurea peaked in urine at 2–4 hr after dosing and was undetectable 12–15 hr after the dose (Dalton et al., 2005). A reduction in dose is recommended for patients with impaired renal function (Yan et al., 2005).

Experimental animal pharmacokinetic data are summarized in Table 14. Data regarding i.p. absorption appear to be reliable in rats and mice. Absorption after i.p. injection appears roughly comparable to oral administration, based on renal excretion data. Metabolism of hydroxyurea to urea occurs in mice, but approximately half the administered drug appears unchanged in the urine. In vitro tests suggest that liver and kidney have the highest capacity for biotransformation of hydroxyurea to urea in mice (Adamson et al., 1965).

Distribution of hydroxyurea to the fetus was shown in rats and monkeys (Wilson et al., 1975) and in rabbits (DeSesso and Goeringer, 1990b). In monkeys i.v. dosed with 100 mg/kg bw/day hydroxyurea on GD 23–32, 27–36, or 31–40, mean maximum maternal blood concentrations were obtained at 1 hr after dosing and measured at levels ranging from ~76–92 μg/mL (Wilson et al., 1975). Hydroxyurea concentration in embryos compared to maternal blood was lower at 4 hr after exposure and higher at 8 and 12 hr after exposure. In rats i.p. injected with hydroxyurea at 100, 137, or 175 mg/kg bw/day on GD 9–12, concentrations of hydroxyurea in embryos exceeded those in maternal blood at ≥1 hr after treatment with 100 mg/kg bw/day and ≥2 hr after treatment with 137 and 175 mg/kg bw/day. In rabbits s.c. injected with 650 mg/kg bw hydroxyurea, embryonic hydroxyurea levels rose steadily over 3 hr, concentrations remained steady from 3–6 hr after treatment, and then concentrations began declining 8 hr after treatment (DeSesso and Goeringer, 1990b). Some studies measured maternal and embryonic half-lives in rats i.p. dosed with up to 250 mg/kg bw/day and monkeys i.v. dosed with 100 mg/kg bw/day (Rajewsky et al., 1971; Wilson et al., 1975). Compared to maternal half-lives, embryonic half-lives for hydroxyurea were two times higher in monkeys and two to six times higher in rats.

2.7.3 General toxicology. The most common adverse effect reported in patients taking hydroxyurea is suppression of bone marrow, which most often results in neutropenia (Bristol-Myers-Squibb, 2004). Thrombocytopenia and anemia can also occur. Hydroxyurea therapy is associated with skin ulceration in patients with myeloproliferative disorders. When skin ulceration occurs in patients with sickle cell disease, it is unclear whether the ulceration is related to the underlying disease or the hydroxyurea.

Bone marrow is a target of toxicity in experimental animals (FDA, 1998a; Gwilt and Tracewell, 1998; Bristol-Myers-Squibb, 1999, 2002, 2004, 2005b). Bone marrow hypoplasia or depression was reported in rats, dogs, and monkeys and leukopenia, macrocythemia, and anemia were reported in unspecified animals. The male reproductive system was also identified as a target in experimental animal studies. Arrested spermatogenesis was observed after exposure to hydroxyurea ≥1260 mg/kg bw/day **[apparently in rats]** and exposure of dogs to ≥140 mg/kg bw/week. Arrested spermatogenesis in dogs was reported to be reversible. Other organs reported to be affected by hydroxyurea exposure included lung (pulmonary congestion and mottling in rats), liver (damage with fatty meta morphogenesis in rats and hemosiderosis in dogs), spleen (lymphoid atrophy in monkeys and hemosiderosis in dogs), and small and large intestine (degeneration in monkeys). Developmental and reproductive toxicity studies are discussed in Sections 3 and 4. A further discussion of effects in spermatogenesis appears in Section 4.2.2.1.

2.7.4 Genetic toxicity. Three studies were found to be acceptable for evaluating genetic toxicity in humans. No increase in lymphocyte chromosome anomalies were observed in eight sickle cell disease patients (aged 7–20 years) who were monitored before hydroxyurea therapy and every 2 months on therapy for 1 year (Khayat et al., 2006). In adults treated with hydroxyurea for myeloproliferative disorders, 7 of 19 (37%) patients untreated previously who had a normal karyotype initially developed clonal abnormalities (Weinfeld et al., 1994); three of six patients treated previously with normal karyotypes at the start of hydroxyurea treatment developed chromosomal abnormalities. Hydroxyurea therapy was not associated with a statistically significant increase in *HPRT* mutant frequency or Vγ-Jβ recombination events in mononuclear cells obtained from 27 adults with myeloproliferative disorder (15 of whom had 0–21 months of hydroxyurea exposure and 12 of whom had 4–18 years of hydroxyurea exposure) or 30 adults with sickle cell disease (15 of whom were exposed to hydroxyurea for a median of 24 months and 15 of whom were unexposed age-matched controls) (Hanft et al., 2000). These studies are limited by lack of control data, small sample, and insufficient length of follow-up.

In drug labels, hydroxyurea was classified as an unequivocal genotoxicant (Bristol-Myers-Squibb, 2005a). Mutagenic activity of hydroxyurea varied according to cell type and locus examined (Martus et al., 1999; IARC, 2000; Bristol-Myers-Squibb, 2005a). In both in vivo and in vitro studies, hydroxyurea induced sister chromatid exchanges, micronuclei, and chromosomal aberrations. Other signs of genetic toxicity observed in in vitro studies included recombination, gene amplification, transformation, and DNA breaks.

2.7.5 Carcinogenicity. Three studies were useful for evaluating risk of carcinogenicity in adults treated with hydroxyurea for myeloproliferative disorders. In one study, nine of 50 adults taking hydroxyurea developed leukemia and one developed a myelodysplastic syndrome Seven of the patients who developed leukemia were treated with hydroxyurea alone (Weinfeld et al., 1994). A second study reported myeloid leukemia or a myelodysplastic syndrome in seven (3.5%) of 201 patients treated with hydroxyurea alone and 14 (5.5%) of 251 patients exposed to hydroxyurea with or without

other agents (Sterkers et al., 1998). About 40% of essential thrombocythemia patients who developed leukemia or a myelodysplastic syndrome on hydroxyurea had a 17p deletion. Najean and Rain (1997) calculated an actuarial risk of leukemia or myelodysplastic syndrome at ~10% by the 13th year of therapy in patients treated with hydroxyurea for polycythemia vera. The risk of other cancer was calculated as ~15% by the 14th year, or about 1.1% annually, which the authors considered to be only slightly greater than the age-adjusted general population rate of 0.8% annually. IARC (2000) concluded that available data did not allow a conclusion on whether the occurrence of acute leukemia or myelodysplastic syndrome in patients treated with hydroxyurea for myeloproliferative disorders represented progression of the myeloproliferative disorder or an effect of treatment.

There are two case reports of leukemia in children on hydroxyurea for sickle cell disease; the short duration of therapy (7 weeks and 6 months) before diagnosis makes a causal relationship less likely (Amrolia et al., 2003). Leukemia has not been reported in adults on hydroxyurea for sickle cell disease, but the longest published follow-up is 9 years (Steinberg et al., 2003), and longer follow-up is needed. Children treated with hydroxyurea are expected to have longer periods of exposure given their longer life expectancy. The underlying diseases (myeloproliferative disorder versus sickle cell disease) may or may not confer different risks of hydroxyurea-associated leukemogenesis.

No adequate animal studies for evaluating carcinogenicity were identified. IARC (2000) concluded that, ''There is *inadequate evidence* in experimental animals for the carcinogenicity of hydroxyurea.'' In their overall evaluation, IARC concluded, ''Hydroxyurea is *not classifiable as to its carcinogenicity to humans (Group 3)*.''

2.7.6 Potential sensitive subpopulations. People with myeloproliferative disorders may be susceptible to possible carcinogenic effects of hydroxyurea. There are no data on possible differences between children and adults in sensitivity to hydroxyurea toxicity. Possible fetal effects are discussed in Section 3.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human

Human developmental toxicity reports on hydroxyurea include use during pregnancy and use during childhood. Reports of hydroxyurea therapy in children focused on the effectiveness of hydroxyurea in the treatment of sickle cell disease and, to a lesser extent, other diseases. The effectiveness of the treatment for a disease is not directly relevant to characterizing the potential toxicity of that treatment, and effectiveness data will not be discussed in detail in this report.

3.1.2 Pregnancy. Hydroxyurea is used for serious illnesses, which may themselves affect pregnancy outcome.

3.1.2.1 Maternal illness and pregnancy: This section includes a brief overview of three diseases for which hydroxyurea may be used in women of child-bearing potential: sickle cell disease, essential thrombocythemia, and chronic myeloid leukemia, with respect to effects of the untreated disease on pregnancy outcome.

According to the American College of Obstetricians and Gynecologists (ACOG) (2005), pregnancy in women with sickle cell disease is associated with increased morbidity and mortality. **[ACOG does not specify a comparison of pregnant women with sickle cell disease to nonpregnant women with sickle cell disease or to pregnant women who do not have sickle cell disease. The statement may be valid for both comparisons.]** Maternal complications include preterm labor and postpartum infection. Fetal complications include intrauterine growth restriction and prematurity.

Pregnancy outcomes from published studies are listed in Table 15. The Sun et al. (2001) study was a retrospective review of 20 years' obstetric records. The Smith et al. (1996) study prospectively enrolled women with sickle cell disease in adulthood. The Serjeant et al. (2004) study followed a cohort of girls with hemoglobin S from birth, perhaps explaining the higher incidence of spontaneous abortion documented in this study.

Table 15
Pregnancy Outcomes in Women with Sickle Cell Disease

	Study		
Endpoint	Smith et al. (1996)	Sun et al. (2001)	Serjeant et al. (2004)
Number of subjects	445 (72% SS, 17% SC, 11% sickle thalassemia)	127 (54% SS, 46% SC)	94 pregnancies in 52 women (SS)
Controls	Historical	129 AA	157 pregnancies in 68 women (AA)
Painful crises	50% of SS, 26% of SC	48% of SS, 19% of SC	
Elective abortion	29%	Not evaluated	11% SS, 8% AA
Spontaneous abortion	6.5%	7% SS, SC not evaluated	36% SS,[a] 10% AA
Stillbirth	0.7%	4% of SS, 2% of SC	7% SS,[a] 0.7% AA
Mean gestational age, weeks	37.5 SS, 38.6 SC	Not reported	37.0 SS,[a] 38.7 AA
Premature (<37 weeks)	27% of SS, 17% of SC	45% of SS,[a] 22% of SC[a]	44% SS,[a] 15% AA
Mean birth weight, g	2650 SS, 3060 SC	—	2500 SS,[a] 3000 AA
Low birth weight (<2500 g)	38% of SS, 17% of SC	46% of SS,[a] 17% of SC	42% SS,[a] 19% AA
Pyelonephritis	—	7% of SS, 5% of SC	—
Preeclampsia	—	10% of SS, 3% of SC	—
Postpartum infection	—	22% of SS,[a] 10% of SC[a]	—
Maternal mortality	—	—	2% SS, 0 AA

[a]Statistically different from controls.
AA, normal hemoglobin; SS, homozygous sickle cell anemia; SC, hemoglobin SC disease.

Treatment of sickle cell disease in pregnant women is similar to its treatment in nonpregnant women. ACOG (2005) recommends folic acid (4 mg/day) for women with sickle cell disease who are contemplating pregnancy. ACOG states that prophylactic transfusion has not been shown to be beneficial in pregnant women with sickle cell disease, and that hydroxyurea treatment during pregnancy has not been studied. ACOG further states that ''the use of hydroxyurea is not recommended during pregnancy because it is teratogenic.'' **[No reference was provided for the statement.]**

Essential thrombocythemia is a myeloproliferative disorder characterized by persistent elevation of the platelet count in the absence of other myeloproliferative or myelodysplastic disorders. Hydroxyurea has been considered the drug of choice for this condition in nonpregnant individuals (Finazzi and Harrison, 2005), although such use of hydroxyurea is off label. According to a review, 280 pregnancies in women with essential thrombocythemia have been reported (Finazzi and Harrison, 2005). Pregnancy complications identified in reviews are listed in Table 16. Recommendations for treatment of essential thrombocythemia during pregnancy include aspirin, interferon-α, and heparin (Tefferi and Murphy, 2001; Finazzi and Harrison, 2005).

Chronic myeloid leukemia is uncommon during pregnancy (Pejovic and Schwartz, 2002; Hurley et al., 2005). Concerns about untreated or inadequately treated chronic myeloid leukemia center on the potential thrombosis and pregnancy loss that may be associated with leukocytosis, which are analogous to the problems reported with essential thrombocythemia (Griesshammer et al., 1998).

3.1.2.2 Hydroxyurea treatment during pregnancy There are no controlled studies on the use of hydroxyurea during human pregnancy.

Thauvin-Robinet et al. (2001), support not indicated, presented 32 pregnancies in 31 women treated with hydroxyurea. Treatment during the first trimester occurred in 22 of the pregnancies. Twenty-two women were treated for essential thrombocythemia, six for chronic myeloid leukemia, two for chronic myeloid splenomegaly, and one for sickle cell disease. Hydroxyurea doses ranged from 500–6000 mg/day. There were one spontaneous and five induced abortions. Two pregnancies were marked by intrauterine growth restriction and nine by premature delivery. There were no major malformations among the offspring. Three minor malformations included pilonidal sinus, dilated ureter, and hip dysplasia. The authors believed the incidences of growth restriction and prematurity to be increased in this population but could not determine if these complications were due to the hydroxyurea therapy or the underlying maternal illness.

Additional case reports and small series are summarized in Table 17.

Strengths/Weaknesses: The report of Thauvin-Robinet (2001) includes 32 pregnancies in 31 women, which is a reasonable number for an assessment. The rest of the studies in Table 17 are single case reports or small case series. Taken as a group, only limited conclusions can be drawn from these cases. Most cases went well and there were no apparent teratogenic effects. Of the 10 outcomes that were not normal, three were elective abortions, one was a stillbirth that appears to have been due to eclampsia, one was a stillbirth of undocumented cause, two were preterm deliveries, two were cases of intrauterine growth restriction, and one outcome was unknown (and may have been normal). Of the two cases with intrauterine growth restriction, one was exposed only at conception and one was a case in a woman with sickle cell disease, which itself is associated with intrauterine growth restriction. The unexplained stillbirth occurred at 33 weeks gestation to a woman who discontinued hydroxyurea at 7 weeks, making it unlikely that the medication was involved in the adverse outcome. Weaknesses of this group of case reports and series include an inability to exclude the underlying maternal illness or exposure to other medications as causes of adverse outcome and the lack of long-term follow-up of gestationally exposed children.

Utility (Adequacy) for CERHR Evaluation Process: These case reports and series taken together are useful in suggesting that use of hydroxyurea during pregnancy is not commonly associated with adverse short-term outcomes.

3.1.3 Sickle cell disease in children

3.1.3.1 Childhood disease and development: This section includes a brief overview of the effects of sickle cell disease on development in children; the references cited in this section are representative of a much larger literature. Children with sickle cell disease have a hemolytic anemia and are at risk for: acute splenic sequestration crisis (massive splenomegaly from trapped blood, associated with a precipitous fall in hematocrit); splenic infarction (which is almost universal by early childhood); infection (from asplenism); aplastic crisis (marrow suppression usually associated with parvovirus B19 infection); acute chest syndrome (pulmonary infiltrate or scan abnormality associated with infection, infarction, or both); stroke (from obstruction of the cerebral circulation); gallbladder disease with pigment gallstones (from chronic hemolysis); as well as chronic, recurring episodes of pain (Wethers, 2000). A prospective study of 694 infants enrolled before the age of 6 months found that children with sickle cell anemia, with or without α-thalassemia, had rates of painful events of 28–43 per 100 person-years and rates of acute chest syndrome of 20–27 per 100 person-years (rounded) (Gill et al., 1995). Stroke occurred at a rate of 1.15 per 100 person-years.

Children with sickle cell disease experience slower growth than children in the general population, with height and weight for age depressed 0.7–2 SD compared to reference populations (Platt et al., 1984; Thomas et al., 2000; Silva and Viana, 2002). These children have a deficit in fat mass and fat-free mass that is attributed to

Table 16
Pregnancy Complications in Women with Essential Thrombocythemia

Complication	Frequency (%)[a]
First trimester loss	26–37
Late pregnancy loss	5–10
Intrauterine growth restriction	4–5
Preterm delivery	6–8
Placental abruption	3
Venous thrombosis	Not given
Transient ischemic attack	Not given
Preeclampsia	Not given

From Finazzi and Harrison (2005) and Tefferi and Murphy (2001).
[a]Percentages rounded to nearest whole number.

Table 17
Exposure to Hydroxyurea in Human Pregnancy

Hydroxyurea treatment				
Gestational age	Dosage	Maternal illness; other exposures	Outcome	Reference
17 weeks	8 g i.v. × 1	Acute myeloid leukemia; cytosine arabinoside, vincristine, 6-thioguanine, prednisone, cephalothin, gentamicin, carbenicillin	Elective abortion; ''no external defects or gross abnormalities in organogenesis''	Doney et al. (1979)
27 weeks	8 g i.v. × 1	Acute myeloid leukemia; cytosine arabinoside, vincristine, 6-thioguanine, prednisone, cefazolin, gentamicin, carbenicillin, amphotericin B, trimethoprim, sulfamethoxazole	Premature delivery at 31 weeks; neonatal hyponatremia, hypocalcemia, hypoglycemia; weight, height, and head circumference below 3rd percentile at 13.5 months; normal Denver Developmental Screening Tests; normal neonatal blood counts	Doney et al. (1979)
Throughout pregnancy	500–1000 mg/day	Chronic myeloid leukemia	Premature delivery at 36 weeks. Normal 2670 g boy with normal blood counts; normal development at 26 months of age	Patel et al. (1991)
Throughout pregnancy	1500 mg/day	Chronic myeloid leukemia	Eclampsia at 26 weeks gestation, stillborn infant without reported malformations	Delmer et al. (1992)
Throughout pregnancy	1500 mg/day	Chronic myeloid leukemia	Normal 3200 g boy born at term	Delmer et al. (1992)
Throughout pregnancy	1000–3000 mg/day	Chronic myeloid leukemia; 1.5 Gy radiation therapy to spleen at 15 weeks gestation	Normal 3100 g boy delivered at term, normal blood counts	Tertian et al. (1992)
Throughout pregnancy	1500–3000 mg/day	Chronic myeloid leukemia	Normal 2850 g girl delivered at 37 weeks, normal blood counts, development normal at 5 months of age	Jackson et al. (1993)
Possibly throughout pregnancy	3–5 capsules	Chronic myeloid leukemia; allopurinol	Normal child, normal blood counts, followed to 6 weeks of age	Szántó and Kovács (1993)[a]
Mid-pregnancy to 1 month before term	Not stated	Chronic myeloid leukemia; leukapheresis	Normal 3400 g boy born at term	Fitzgerald and McCann (1993)
Conception until 6 weeks gestation	1000–2100 mg/day	Essential thrombocythemia	Normal 6 lb **[~2700 g]** boy delivered at ''35 weeks'' of pregnancy **[probably 37 weeks]**, normal blood counts	Cinkotai et al. (1994)
Not stated	Not stated	Sickle cell disease	Elective abortion	Charache et al. (1995)
Not stated	Not stated	Sickle cell disease	Elective abortion	Charache et al. (1995)
Not stated	Not stated	Sickle cell disease	Normal child born at term	Charache et al. (1995)
18–28 weeks	500–1000 mg/day	Essential thrombocythemia	Normal 2970 g boy born at term	Dell'Isola et al. (1997)[b]
Conception to 9 weeks gestation	1000 mg/day	Sickle cell disease; folic acid	Normal 3240 g boy	Diav-Citrin et al. (1999)
Not stated	Not stated	Sickle cell disease	Normal outcome	de Montalembert et al. (1999)
Conception to 5 weeks gestation	1000 mg/day	Sickle cell anemia; folic acid, hydrocodone, iron, amoxicillin	Normal 2750 g boy delivered at 37 weeks, mild respiratory distress, lactose intolerance, normal blood counts; normal development at 17 months of age	Byrd et al. (1999)

Table 17
Continued

Hydroxyurea treatment				
Gestational age	Dosage	Maternal illness; other exposures	Outcome	Reference
Conception to 4 weeks gestation	500 mg/day	Sickle cell anemia; folic acid, ranitidine, penicillin, doxepin, albuterol, hydrocodone	Intrauterine growth restriction by ultrasound with iatrogenic preterm delivery at 32 weeks of 1,365 g boy; respiratory distress, apnea, bradycardia, hyperbilirubinemia, patent ductus arteriosus, sepsis; normal development at 21 months of age	Byrd et al. (1999)
19–38 weeks	1000–1500 mg/day	Chronic myeloid leukemia	Normal 3400 g girl delivered at 38 weeks	Celiloglu et al. (2000)
18–28, 34–37 weeks gestation	1500 mg/day	Chronic myeloid leukemia; allopurinol, α-interferon	Delivery at 37 weeks of normal 2450 g girl with normal blood counts	Baykal et al. (2000)
Throughout pregnancy	Not stated	Essential thrombocythemia	Normal outcome	Wright and Tefferi (2001)
27–38 weeks	1500–4000 mg/day	Chronic myeloid leukemia	Normal 2680 g boy delivered at term; normal blood counts	Fadilah et al. (2002)
Conception to 7 weeks	500 mg/day	Essential thrombocythemia; platelet pheresis, aspirin	Intrauterine fetal death at 33 weeks	Koh et al. (2002)
Discontinued "at conception"	Not stated	Essential thrombocythemia; aspirin (2nd pregnancy in the patient presented above)	Labor induced at 35 weeks for decreased fetal growth and increased umbilical artery resistance indices: normal 1940 g girl	Koh et al. (2002)
Conception to 9 weeks	Not stated	Polycythemia vera	Normal 3550 g boy delivered at 37 weeks; development normal at 12 months of age	Pata et al. (2004)
Not stated	Not stated	Sickle cell disease	Not stated **[possibly the normal outcome reported previously by de Montalembert et al. (1999)]**	de Montalembert et al. (2006)

[a]This study is in Hungarian. Information was taken from the English abstract.
[b]This study is in Italian. Information was taken from the English abstract and unofficial translation.

increased energy expenditure and a delay in skeletal maturation (Barden et al., 2002). Puberty is delayed in children with sickle cell disease (Platt et al., 1984; Barden et al., 2002; Serjeant et al., 2004), due probably to the lower weight and body fat compared to children without hemoglobinopathies. Pubertal progression is delayed in children with sickle cell disease; the delay is attributable to the lower weight for age and is not indicative of gonadal dysfunction (Platt et al., 1984).

3.1.3.2 Hydroxyurea treatment for hematologic disorders during childhood: Based on theoretical benefits of elevated hemoglobin F in sickle cell disease and on experience in adults (Charache et al., 1995), several centers have used hydroxyurea therapy in children with sickle cell disease. There are a few additional reports on the use of hydroxyurea for polycythemia or thalassemia.

Cornu (1994), support not indicated, presented a retrospective report on 170 patients with congenital cyanotic heart disease. The patients had been referred for hematologic consultation due to polycythemia secondary to hypoxemia. The approach of the author's group was to give hydroxyurea or pipobroman to suppress marrow production of erythrocytes. The report involved children and adults aged between 6 months to 57 years at the start of therapy. There were 17 patients younger than 10 years of age. **[None of the results were broken down by age.]** Hydroxyurea was given initially at 10 or 15 mg/kg bw/day. The goal of therapy was to maintain a hematocrit near 60% and a mean corpuscular hemoglobin concentration of 35 g/dL. Phlebotomy was used for hematocrit >65%. The author compared laboratory and clinical results of therapy between patients starting therapy with a hematocrit >65% and those starting therapy with a hematocrit <65%. **[This comparison is not further discussed here.]**

Therapy was described as causing a decrease in erythrocyte count and increases in mean corpuscular hemoglobin concentration and mean corpuscular volume. **[Laboratory data from baseline and during therapy were shown for a subset of 78 patients currently on therapy. Statistical analysis was not reported by the author. Student's *t*-test by CERHR showed a statistically significant decrease in erythrocyte count and a statistically significant increase in mean corpuscular volume. A 3% increase in mean corpuscular hemoglobin concentration was significant at $P = 0.0549$.]** The author also reported improved functional capabilities, with 74% of patients returning to normal activities. Therapy was limited by thrombocytopenia, which prevented an optimum dose of chemotherapy from being reached or maintained in 54 of the initial 170 patients. The author also indicated that gastrointestinal and cutaneous side effects occurred, but that they disappeared within a few weeks.

There were 39 deaths, 24 of which were attributed to cardiac insufficiency or sudden death. Acute intercurrent events were responsible for 15 deaths and included heart-lung transplant complications, lung disease, anemia, encephalitis, cerebral embolism, cerebral hematoma, brain abscess, and massive hemoptysis. The author concluded that marrow suppression therapy was an effective and well tolerated treatment of polycythemia associated with congenital cyanotic heart disease. The author recommended that therapy be started in early childhood for optimum protection against treatment-associated thrombocytopenia.

[Another study from this group (Triadou et al., 1994), published the same year, presented laboratory data from 64 patients ages 8–47 years who were treated with hydroxyurea for cyanotic congenital heart disease. It is not known whether the same patients were used for both studies.]

Strengths/Weaknesses: Strengths of this study are the large sample size and the use of functional capacity as an outcome measure. Weaknesses are the wide age range, the lack of results by age, failure to reach optimum hydroxyurea dose in 54/170 patients, and the numerous co-morbidities in these patients.

Utility (Adequacy) for CERHR Evaluation Process: This report is not useful to the evaluation because of the lack of age-specific information and the presence of confounding co-morbidity. In addition, polycythemia as a result of congenital cyanotic heart disease is rarely seen due to early diagnosis and surgical intervention.

Ferster et al. (1996), supported by the Belgian National Fund for Scientific Research, enrolled 25 children and young adults in a randomized, single-blind, placebo-controlled cross-over trial of hydroxyurea for severe sickle cell anemia. The subjects ranged in age from 2–22 years, with a median age of 9 years. Hydroxyurea was administered at 20 mg/kg bw/day for 2 months, after which hydroxyurea was given at 25 mg/kg bw/day if there was $<2\%$ increase in hemoglobin F levels. The hydroxyurea dose was decreased by 50% for neutropenia (leukocyte count $<3\times10^9$/L) or thrombocytopenia (platelets $<8\times10^9$/L). After 6 months on hydroxyurea or placebo, subjects were switched to the opposite treatment. The planned endpoints were the number of hospitalizations and the number of days in the hospital. Data were analyzed by Wilcoxon rank-sum test.

There were 22 evaluable subjects after excluding three patients who did not attend their evaluation visits. Laboratory data are summarized in Table 18. Three subjects on placebo and 16 subjects on hydroxyurea were not hospitalized during the 6-month treatment period. The authors stated that ''no clinically relevant toxicity'' was associated with hydroxyurea therapy. The authors concluded that treatment with hydroxyurea produced a clear clinical benefit.

Strengths/Weaknesses: The use of a placebo control, a population purely with hemoglobin SS and severe clinical disease, standardized dosing criteria, and predefined toxicity criteria are strengths. No decreases in dose were needed because hematologic side effects were mild, and no other side effects were noted. Weaknesses are the small sample size and the lack of long-term outcome data.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful.

Ferster et al. (2001), supported by the Belgian National Fund for Scientific Research, published results from a national registry of 93 hydroxyurea-treated children and young adults with sickle cell disease. Subjects had been hospitalized at least twice for sickle cell disease-related events in the year before starting hydroxyurea therapy. The initial hydroxyurea dose was 20 mg/kg bw/day, with increases of 5 mg/kg bw/day at the discretion of the patient's physician. No attempt was made to reach a maximum tolerated dose, and nearly all patients were on $\leq$25 mg/kg bw/day at the end of the first year of therapy. Comparisons of hematologic test results and days of hospitalization were made between years of therapy using Student's *t*-test with Bonferroni adjustment and Wilcoxon signed rank test and between first and fifth years of therapy (for patients with 5 years of experience) using analysis of variance (ANOVA). Kaplan-Meier survival curves were used to evaluate the time to the first vaso-occlusive crisis or other event on therapy.

The median age at the beginning of therapy was 7 years (range = 8 months to 45 years). There were 82 patients at the 1-year evaluation, 61 at 2 years, 44 at 3 years, 33 at 4 years, 22 at 5 years, and 12 at 6 years. There were reductions in hospitalizations and sickle cell disease-related events after 1 year, but there was no additional effect of subsequent years of therapy; that is, the apparent beneficial effects of therapy were maintained at the same level through 5 years of therapy. The cumulative probability of not experiencing a sickle cell disease-related event after 5 years of hydroxyurea therapy was 47%. Changes in hematologic endpoints after 1 year of therapy are summarized in Table 19. No additional changes occurred in subsequent years. There were no deaths, leg ulcers, or recurrent strokes in patients on hydroxyurea. Nail, skin, and hair changes were not reported, leading the authors to conclude that if

Table 18
Changes in Laboratory Values in Subjects on Hydroxyurea for Sickle Cell Anemia

Endpoint	Comparison to placebo
Hemoglobin	↔
Mean corpuscular volume	↑12%
Mean corpuscular hemoglobin concentration	↔
Platelet count	↔
Leukocyte count	↓29%
Hemoglobin F	↑3.3-fold
Reticulocyte count	↓31%

From Ferster et al. (1996).
↑,↓,↔, Statistically significant increase, decrease, or no change, respectively.

Table 19
Changes in Hematologic Endpoints in the Belgian Hydroxyurea Registry

Laboratory test	Change compared to baseline
Hemoglobin	↑7%
Percent hemoglobin F	↑2.3-fold
Mean corpuscular volume	↑13%
Neutrophil count	↓35%

From Ferster et al. (2001).
↑,↓, Statistically significant increase or decrease, respectively.

Table 20
Laboratory Data in Children Treated with Hydroxyurea

Endpoint	Value at maximum dose compared to pre-treatment value
Fetal hemoglobin	↑4.6-fold
Mean corpuscular volume	↑24%
Hemoglobin	↑18%
Platelet count	↓20%
Leukocyte count	↓31%
Absolute neutrophil count	"↓" 31%[a]
Reticulocyte count	↓30%
Total bilirubin	↓25%
Lactate dehydrogenase	↓16%

From Jayabose et al. (1996).
[a]Value characterized by the authors as decreased, although statistical significance not attained ($p = 0.12$).
↑,↓, Statistically significant increase or decrease, respectively, compared to pre-treatment value.

such changes occurred, they were considered minor by the physicians reporting to the registry. The authors concluded that prolonged hydroxyurea treatment of young patients with sickle cell disease appears efficacious, safe, and cost-effective.

Strengths/Weaknesses: The large sample size, long-term follow-up (in a sub sample), and comparison of hematologic endpoints to baseline are strengths. Weaknesses include the lack of fixed guidelines for hydroxyurea dose escalation, the different genotypes of the sickle cell disease patients, failure to reach maximum tolerated dose, and the continued use of transfusion in some patients, which may have confounded hematologic results. Although the median age at the beginning of therapy was 7, the oldest patient was 45 years old, and six patients were >20 years old. It was not clear whether attrition was responsible for there being only 22 patients left at 5 years and 12 left at 6 years or whether patients entered the study at different times.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for assessment of the long-term outcome of hydroxyurea therapy in children.

Jayabose et al. (1996), supported in part by Healix Healthcare Inc., conducted an open label pilot study to examine efficacy and toxicity of hydroxyurea for treatment of sickle cell anemia in children. Although the focus of this study was efficacy as determined by number of vaso-occlusive crises, this discussion will focus on laboratory test results and toxicity effects. The subjects in this study included 10 male and five female children with a median age of 15.3 years (range = 4.2–18.8 years). The children had sickle cell anemia with frequent vaso-occlusive crises or severe anemia. Dosing was started at 20 mg/kg bw/day and increased by increments of 5 mg/kg bw over 4–8 weeks unless limited by neutropenia or thrombocytopenia. Doses did not exceed 35 mg/kg bw or 2000 mg/day. A diary system was attempted to monitor compliance but was not used by many subjects. Blood cell counts, reticulocyte counts, fetal hemoglobin levels, and clinical chemistry endpoints were monitored at baseline; at 2, 4, and 8 weeks of treatment; and after dose increases. Data were analyzed by Student's *t*-test and χ^2 test. Rates of adverse events were based on total events in all subjects divided by subject-years, and post-hydroxyurea events were compared to the pre-hydroxyurea experiences of the subjects.

Table 21
Changes in Laboratory Values in Children on Hydroxyurea

Endpoint	Change from pre-treatment value
Hemoglobin	↑16%
Mean corpuscular volume	↑18%
Percent hemoglobin F	↑2.2-fold
Reticulocyte count	↔
Bilirubin	↑36%

From Scott et al. (1996).
↑,↔, Statistically significant increase or no change, respectively.

There were 14 evaluable patients in this study; one child had to discontinue treatment due to severe nausea. Results of laboratory testing are outlined in Table 20. The study authors noted that although mean platelet and leukocyte counts were decreased significantly during treatment, there was no significant effect on absolute neutrophil count. Other endpoints listed in Table 20 were related more to efficacy than to toxicity. In addition to nausea observed in one child, a second child experienced mild hair loss that resolved after the dose was reduced from 23.5 to 18.75 mg/kg bw. A third patient had asymptomatic neutropenia (absolute neutrophil count of 0.84×10^9/L), which resolved without a change in dose; the same patient had varicella, which was treated with acyclovir. Thrombocytopenia was not observed in any patient. The study authors concluded that a hydroxyurea dose of 20–35 mg/kg bw increases fetal hemoglobin in most patients without inducing serious toxicity. The authors noted that more studies were needed to show efficacy and long-term safety.

Strengths/Weaknesses: Strengths include the involvement of children only (mostly teenagers) and the comparison of hematologic results to baseline. Weaknesses are the small size of the study, the open-label design, and the differences in maximum tolerated doses.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Scott et al. (1996), support not indicated, reported on 15 children between 10 and 17 years old who were given hydroxyurea for severe sickle cell disease characterized by at least three hospitalizations/year for acute painful events. Hydroxyurea was given at a starting dose of 10–20 mg/kg bw/day and was increased at 12-week intervals by 5 or 10 mg/kg bw/day if there was no toxic reaction. Doses were reduced if defined decreases in hematologic cell counts occurred, and hydroxyurea was held until recovery if predetermined thresholds were reached (neutrophils $<2000/mm^3$, reticulocytes $<80{,}000/mm^3$, hemoglobin <4.5 g/dL or 20% less than the starting value, platelets $<80{,}000/mm^3$). Patients received folic acid 1 mg/day. Statistical comparisons were made between values obtained before hydroxyurea therapy and on therapy using the paired Student's *t*-test. Of the 15 patients, 13 completed at least 6 months of therapy and were considered evaluable. Median follow-up was 24 months (range = 6–39 months) and the mean ± SD hydroxyurea dose was 22.8 ± 6.0 mg/kg bw/day (range = 14.1–34.7 mg/kg bw/day). Changes in laboratory values are summarized in Table 21.

In the eight children who completed at least 2 years of therapy, height and weight percentiles were maintained. One child, who had been below the 5th percentile for height and weight, reached the 5th percentile for height and the 25th percentile for weight. The authors remarked that the subjects appeared to progress normally through puberty. **[No data were shown.]** There were three episodes of cytopenia on therapy. In one case, parvovirus B19 infection was suspected based on high antibody titers. In all three episodes, hydroxyurea therapy was resumed after recovery without further difficulty. One child died of a hemorrhagic stroke that appeared unrelated to hydroxyurea therapy based on the results of laboratory studies. A statistically significant reduction in hospitalization for vaso-occlusive crisis was seen in subjects who were on hydroxyurea therapy for at least 1 year. The authors concluded that hydroxyurea treatment appeared to improve the hematologic status of most patients studied and that their preliminary data provided a compelling reason to carry out a randomized controlled trial of hydroxyurea in children.

Strengths/Weaknesses: The reporting of growth and development in teenagers on hydroxyurea and the assessment of compliance are strengths not seen in many other studies. Weaknesses are the small sample size, inclusion of three different sickle cell genotypes, the wide range of follow-up durations, and the lack of presentation of data on pubertal progression.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Rogers (1997), support not indicated, reported on 16 children with sickle cell disease treated with hydroxyurea for 6–50 months. The children's ages ranged from 5.3–18.4 years. Hydroxyurea was started at 15 mg/kg bw/day and increased at 8-week intervals by 5 mg/kg bw/day in the absence of toxicity (based primarily on neutrophil counts). The target dose was 35 mg/kg bw/day. Frequency of clinical events before and during therapy was compared using the Wilcoxon ranked-sum test. The number of admissions and days of hospitalization for painful events decreased by one-third. The number of transfusions also decreased on therapy. There was an 80% reduction in the frequency of acute chest syndrome. The mean percent hemoglobin F increased 5.6-fold at maximum response (9 months of therapy), although it fell back to a 3.6-fold increase compared to baseline at the time of the report. The range of hemoglobin F response was wide, spanning an order of magnitude. Total hemoglobin and mean corpuscular volume increased by about 30% on therapy. Height and weight data were collected from 15 patients, 10 of whom grew an average of 7 cm during treatment. Growth velocity was described as normal. **[No data were shown.]** The other five patients were characterized as older and at their adult height when treatment began. All patients gained weight during treatment. Eight patients developed neutropenia, characterized as a neutrophil count $<2000/\mu L$. Neutrophil counts returned to normal when hydroxyurea was withheld for 1–4 weeks. Two patients developed pigmented nails, and one patient developed oral ulcers. The author concluded that hydroxyurea appeared to have the same effectiveness for sickle cell disease in children as in adults, but that long-term safety concerns had not been resolved.

Strengths/Weaknesses: The detailed study of growth is a strength but is offset partly by the failure to show the growth data. Other weaknesses include the small sample size, the inclusion of multiple sickle cell disease genotypes, the lack of predefined hematologic toxicity criteria, the failure to reach the maximum planned hydroxyurea dose, the use of blood transfusion in some patients, and the lack of statistical analysis of changes from baseline.

Utility (Adequacy) for CERHR Evaluation Process: This study is of utility in the evaluation.

Oury et al. (1997), support not indicated, reported eight children, ages 5–16 years, who were given hydroxyurea for severe sickle cell disease (6 children with sickle cell anemia and 2 children with sickle-β thalassemia). The initial hydroxyurea dose was 15 mg/kg bw/day, which could be raised in increments of 5 mg/kg bw/day to achieve optimum response. All but one child were on therapy for at least 6 months, and the mean duration of therapy was 10 months. Hydroxyurea doses at the end of the study ranged from 14–27 mg/kg bw/day. Hemoglobin F level, monthly blood transfusion, mean number of days/month of hospitalization for vaso-occlusive crises, and pain intensity of crises using a visual analog scale were compared to pre-treatment values for each subject using at least 1 year of pre-treatment experience. No statistical testing was carried out. The authors stated that one subject left the study after 3 months because of the development of idiopathic thrombocytopenic purpura. **[No details were given on this diagnosis.]** The other subjects experienced increased hemoglobin F concentrations, decreased hospitalizations for vaso-occlusive crisis, and decreased pain intensity. The authors indicated in their Discussion section that they did not see evidence of myelotoxicity, but no data were presented on hematologic values other than hemoglobin F percentages. The authors concluded that hydroxyurea at doses lower than those that are myelotoxic can be effective in the treatment of symptomatic sickle cell disease, but that information on possible secondary effects of long-term treatment was lacking.

Strengths/Weaknesses: Weaknesses of this study include the small sample size, the short-term follow-up, the lack of statistical evaluation, and the paucity of data. More information on the child with idiopathic thrombocytopenic purpura is needed.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful.

de Montalembert et al. (1997), supported by the European Union, reported on hydroxyurea treatment of 35 young people with sickle cell disease. The patients ranged in age from 3–20 years (16 were younger than 11 years, 13 were 11–17 years old, and 6 were older than 17). The children were given hydroxyurea 20 mg/kg bw/day 4 days/week, with an increase of 5 mg/kg bw/day every 4 weeks if toxicity did not occur. Defined hematologic criteria were used to identify myelotoxicity and to temporarily stop hydroxyurea treatment. Hydroxyurea was also stopped if there was severe infection, a vascular accident, or worsening anemia requiring transfusion. Treatment durations ranged from 12–59 months. The mean hydroxyurea dose after 6 months of treatment was 33–34 mg/kg bw/day. Laboratory values at 1, 2, and 3 years of therapy were compared to baseline values using

Table 22
Change in Laboratory Values in Children After 1 Year of Hydroxyurea Therapy

Laboratory value	Change from baseline value (%)
Hemoglobin	↑7
Neutrophil count	↓42
Platelet count	↔
Reticulocyte count	↓41
Alanine aminotransferase	↔
Bilirubin	↔
Creatinine	↔

From de Montalembert et al. (1997).
↑,↓,↔, Statistically significant increase, decrease, or no change, respectively.

the paired Student's *t*-test. Number of hospital days for painful events was compared between the study period and the year before the study period. Growth was evaluated using *z*-scores separately in three age groups: 4–11 years, 11–17 years, and >17 years.

Mean hemoglobin F levels peaked after 9 months of treatment at 3.9 times the baseline mean, with large variability in the individual measurements. Other laboratory findings are summarized in Table 22. No significant additional changes occurred in Study years 2 or 3 except for a 20% increase in neutrophil count between Study years 1 and 2.

There was no evidence of hepatic or renal toxicity of hydroxyurea, although one girl developed renal failure attributed to systemic lupus erythematosus on the basis of serology and renal biopsy studies. Growth velocity, assessed by *z*-scores at baseline, 1, and 2 years, was not changed in any age group. The authors stated that "no anomaly of sexual maturation was reported." **[They did not state whether or how pubertal progression was systematically evaluated.]** There was hair loss in one patient and nail hyperpigmentation in five patients, but none left the study due to these side effects. Except for two children, all patients reported a decrease in painful episodes on hydroxyurea therapy. The authors concluded that there was good short- and middle-term tolerance of hydroxyurea but cautioned that long-term outcome data were not available.

Strengths/Weaknesses: The long-term follow-up and standard growth velocity assessment are strengths of this study. Weaknesses are the inclusion of multiple sickle cell disease genotypes and the lack of systematic evaluation of pubertal progression.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Maier-Redelsperger et al. (1998), supported by French government agencies and the European Union, studied the use of hydroxyurea in 29 young patients with sickle cell disease. These subjects were part of a study reported previously (de Montalembert et al., 1997). The mean age of the subjects was 10.9 years (range = 5–19 years). Subjects had been hospitalized at least three times for painful crises in the previous year. Subjects were given hydroxyurea for a mean duration of 22 months (range = 12–36 months). The medication was started at 20 mg/kg bw/day on each of 4 consecutive days/week with an increase of 5 mg/kg bw/day each month to a maximum of 40 mg/kg bw/day. Hydroxyurea was not increased if there was evidence of myelotoxicity, and the medication was stopped temporarily at predetermined thresholds (reticulocytes $<50\times10^9$/L, neutrophils $<1.5\times10^9$/L, or platelets $<100\times10^9$/L). Comparisons of laboratory values were made with pretreatment values at 1, 2, and 3 years and at the time of maximum hemoglobin F response. Statistical analysis was carried out using the Wilcoxon signed rank test. **[Other analyses were carried out on subgroups of patients to identify predictors of therapeutic effectiveness; these analyses are not discussed here.]**

Table 23
Laboratory Values at Time of Maximum Hemoglobin F in Children on Hydroxyurea

Measure	Comparison to pre-treatment value
Total hemoglobin	↑6%
Mean corpuscular volume	↑20%
Neutrophil count	↓42%
Platelet count	"↓"13%[a]
Reticulocyte count	↓45%
Hemoglobin F	↑4.3-fold
Reticulocytes containing hemoglobin F	↑3.1-fold
Cells containing hemoglobin F	↑2.5-fold
Hemoglobin F/cell containing hemoglobin F	↑2.1-fold

From Maier-Redelsperger et al. (1998).
[a]"↓" Decrease identified by the authors, although not statistically significant.
↑,↓, Statistically significant increase or decrease, respectively.

The mean final dose was 34.2 ± 4.6 mg/kg bw/day **[error not identified]**. Treatment was stopped in two children who were believed to have not responded to therapy and in one child who developed systemic lupus erythematosus. A fourth child moved from the area. The changes in laboratory values at time of maximum hemoglobin F response are summarized in Table 23. The authors found that hemoglobin F increased in all but one subject and peaked after 6–18 months of therapy. Thereafter, hemoglobin F was maintained at slightly lower than maximum levels. Adverse effects of therapy were not addressed specifically in this study, which focused on the cellular and molecular responses to hydroxyurea. The authors concluded that the hemoglobin F response to hydroxyurea was sustained at a level slightly lower than the maximum hemoglobin F value and was dependent on the initial hemoglobin F value.

Strengths/Weaknesses: Strengths are the inclusion of a single sickle cell disease genotype, the length of follow-up, and the predefined hematologic criteria for stopping hydroxyurea. It is a weakness that the subjects were part of another study.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

de Montalembert et al. (1999), support not indicated, presented results of a survey sent to French physicians likely to be treating children with sickle cell disease. Information on tolerance of hydroxyurea therapy was presented for 101 children, 23 of whom had been treated for up to 1 year, 33 for up to 1–2 years, 9 for 2–3 years, 14 for 3–4 years, 8 for 4–5 years, and 14 for longer than 5

years. **[No information was given on the number of physicians approached, the response rate, or differences between responders and non-responders.]** The mean ± SD age at onset of hydroxyurea therapy was 9.8 ± 0.4 years (range = 2–20 years). The mean ± SD hydroxyurea dose was 21.4 ± 0.5 mg/kg bw/day (range = 9–30 mg/kg bw/day). Therapy was stopped in 17 children. The most common reasons given for stopping therapy were failure of treatment (n = 6) and relocation of patient (n = 2). There were single instances of treatment stoppage due to non-compliance, pregnancy, rash, leg ulcer, lupus, and acute lymphocytic leukemia. The case of leukemia was diagnosed <2 months after the patient began hydroxyurea and was not believed to have been caused by the therapy. The pregnancy resulted in a normal outcome. **[No information was given on the gestational age at which therapy was stopped.]** There were instances of neutropenia (n = 5), thrombocytopenia (n = 4), and reticulocytopenia (n = 5) that resolved with temporary cessation of hydroxyurea. In addition, seven children developed pigmented nails, three complained of headache, two complained of drowsiness, and a 17-year-old girl developed secondary amenorrhea. In 13 children who began hydroxyurea before age 5 years, weight and height for age were evaluated using *z*-scores, and it was determined that they were not affected by therapy. The authors concluded that hydroxyurea therapy did not cause any pronounced toxicity in children after a median follow-up of 22 months.

Strengths/Weaknesses: Strengths are the large sample size, the single sickle cell disease genotype (except for 2 patients), and the long-term follow-up. Weaknesses are the survey design of the study, without information on number of physicians approached, response rate, or differences between responders and non-responders; the descriptive nature of the study; and the diverse doses of hydroxyurea.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility for the evaluation.

de Montalembert et al. (2006), supported by the French National Institute for Medical Research and the Clinical Research Delegation, reported on 225 children with sickle cell disease who were treated with hydroxyurea at 50 French centers for a median of 3.8 years (range = 1 day to 12.7 years). Seventy-five patients were treated for >5 years, and 10 of those patients were treated for >10 years. The mean age at onset of treatment was 9.2 years (range = 17 months to 19 years). **[The subjects were treated under different protocols, and some were included in previous reports (de Montalembert et al., 1997, 1999; Maier-Redelsperger et al., 1998).]** Patients could be on a hydroxyurea dose as high as 40 mg/kg bw/day, although two-thirds received 15–25 mg/kg bw/day. The dose was given daily in most patients, with some patients receiving hydroxyurea 4 days/week. One patient died at age 18 after receiving hydroxyurea for 11 months. The death was attributed to sickle cell disease-related cardiomyopathy. Hydroxyurea was stopped in 30 patients due to absence of improvement; in 17 due to noncompliance; in five or six due to hypersplenism **[both numbers appear in different parts of the report]**; in three due to elevated transcranial Doppler velocimetry (suggesting an increase in stroke risk); in three due to osteonecrosis of the femoral head; in two each due to stroke, rash, dizziness, and headache; and in one each due to anemia, azoospermia, leg ulcer, planned pregnancy, unplanned pregnancy, middle cerebral artery stenosis, leukemia, systemic lupus erythematosus, sarcoidosis, and use of interferon for hepatitis C. **[Pregnancy outcomes were not provided. One of these pregnancies may have been reported in the previous study (de Montalembert et al., 1999).]** The authors noted that although sickle cell disease is a risk factor for hypersplenism, the hydroxyurea may have been responsible for the hypersplenism in six children in this study, because hydroxyurea can prevent or delay functional asplenia and may permit splenic regeneration. The authors stated that the biggest problems with hydroxyurea therapy for sickle cell disease are treatment failure and non-compliance.

Table 24
Laboratory Values in Children on Hydroxyurea Therapy

Laboratory value	Change from pretreatment period
Hemoglobin	↑15%
Reticulocyte count	↓39%
Percent hemoglobin F	↑2.2-fold
Mean corpuscular volume	↑20%
Neutrophil count	↓34%
Platelet count	↓29%
Percent pitted erythrocytes	↔

From Olivieri and Vichinsky (1998).
↑,↓,↔, Statistically significant increase, decrease, or no change, respectively.

Table 25
Hematologic Values in Children Treated with Hydroxyurea for Sickle Cell Disease

Laboratory value	Comparison at 1 yr with baseline value
Hemoglobin	↑17%
Mean corpuscular volume	↑29%
Mean corpuscular hemoglobin	↑38%
Mean corpuscular hemoglobin concentration	↑10%
Leukocyte count	↓46%
Neutrophil count	↓56%
Platelet count	↓28%
Hemoglobin F	↑6.7-fold

From Koren et al. (1999).
↑,↓, Statistically significant increase or decrease, respectively.

Strengths/Weaknesses: The large sample size is a strength; however, the combining of patients from previous case series resulted in varied doses and duration of hydroxyurea therapy. Other weaknesses include the mixture of sickle cell genotypes, the retrospective nature of adverse-event reporting, and the lack of reporting of hematologic toxicity data.

Utility (Adequacy) for CERHR Evaluation Process: The Expert Panel has little confidence in this study as a source of meaningful data. This study is not useful for the evaluation.

Olivieri and Vichinsky (1998), supported by the University of Toronto, the Medical Research Council of

Canada, the Ontario Heart and Stroke Foundation, and the National Institutes of Health (NIH), reported on 17 children, ages 5–18 years, treated with hydroxyurea for sickle cell disease. Hydroxyurea was started at 7.2–15 mg/kg bw/day, and maximum tolerated doses were 6.7–32 mg/kg bw/day. Patients were followed for a mean ± SEM duration of 18.5 ± 2.1 months. Blood counts were measured serially, and spleen function was assessed by counting erythrocytes that contained endocytic vacuoles (pitted erythrocytes). Comparisons were made between pretreatment laboratory values and values at the end of treatment using paired Student's *t*-test. Laboratory results are summarized in Table 24. There was a decrease in painful crises, blood transfusions, and days in the hospital. Nine patients had temporary cessation of therapy due to neutropenia, and one patient complained of rash, nausea, conjunctivitis, and hair loss. The authors determined that compliance with therapy was excellent based on data collected by a sensor in the caps of the medication bottles. They concluded that spleen function was not altered by 1 year of hydroxyurea therapy.

Strengths/Weaknesses: Strengths are the determination of compliance with objective measures in 10 patients, the assessment of splenic function with objective measures, and the use of predefined criteria for hematologic toxicity. Weaknesses are the small sample size, the use of varied hydroxyurea doses with a low maximum tolerated dose, and the lack of specification of sickle cell disease genotype.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Koren et al. (1999), support not indicated, reported 19 young people treated with hydroxyurea for sickle cell disease. Subject ages ranged from 7–23 years at the beginning of the study, and seven subjects were younger than 15 years. Subjects received hydroxyurea 20 mg/kg bw/day, rounded to permit the use of one or more 500-mg tablets. Patients were followed for 20–66 months, at which time the hydroxyurea dose was 16.4–31.2 mg/kg bw/day. Clinical events were recorded and compared to the incidence of these events during the 2 years before hydroxyurea therapy. Hydroxyurea therapy was held if the neutrophil count was $< 2 \times 10^9$/L, the platelet count $< 80 \times 10^9$/L, or the hemoglobin level < 4.5 g/dL. Comparisons were made using Student's *t*-test.

There were statistically significant decreases in the number of vaso-occlusive crises, blood transfusions, and days in the hospital. Changes in hematologic values are summarized in Table 25. Hematologic toxicity resulted in temporary stopping of hydroxyurea in one subject with anemia and neutropenia. The blood counts recovered over 6 weeks, and hydroxyurea was resumed at a lower dose without further adverse events. Two cases of aseptic necrosis of the hip on hydroxyurea therapy occurred in subjects with previous stroke. Before therapy, four other subjects had had aseptic necrosis of the hip. The authors concluded that the response of children and teenagers to hydroxyurea therapy for sickle cell disease was similar to that of adults and that no severe adverse effects were seen.

Strengths/Weaknesses: Strengths are the predefined criteria for hematologic toxicity and the longer-term follow-up of 2–5 years. The different maximum tolerated doses of hydroxyurea is a weakness, as is the small sample size.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Ware et al. (1999), supported by the Duke Children's Miracle Network Telethon, evaluated the utility of hydroxyurea in the prevention of recurrent stroke in 16 children with sickle cell disease. The children had all been treated with erythrocyte transfusion, the standard therapy for prevention of recurrent stroke in patients with sickle cell disease but were candidates for discontinuing transfusion because of alloantibody formation, intolerance of chelation therapy (to prevent iron overload), or noncompliance with the transfusion regimen. The subjects were 2.9–19.1 years old when transfusion therapy was stopped. Two weeks after transfusion therapy was stopped, hydroxyurea was given at 15 mg/kg bw/day and increased by 5 mg/kg bw/day every 8 weeks to a maximum of 30 mg/kg bw/day. Hydroxyurea was withheld for a hemoglobin concentration < 5 g/dL, neutrophil count $< 1.5 \times 10^9$/L, or platelet count $< 80 \times 10^9$/L. Subjects underwent phlebotomy every 2 weeks with removal of 5–10 mL/kg bw of blood to control iron overload and to stimulate erythropoiesis. No statistical comparisons were made except with regard to the effects of phlebotomy on serum ferritin.

Subjects received hydroxyurea for a mean of 22 months (range = 3–52 months), with a mean ± SD final dose of 24.9 ± 4.2 mg/kg bw/day (range = 19.1–32.7 mg/kg bw/day). Six children had minor painful events while on hydroxyurea; there were no hospitalizations or transfusions for vaso-occlusive crisis. Three subjects had neurologic events consistent with recurrent stroke, for a recurrence rate of 19%. The authors believed that without any treatment, this group of patients would have had a recurrent stroke risk of ~50%. They further stated that the stroke recurrence at their institution for children on prophylactic transfusion therapy was 11%. They concluded that hydroxyurea therapy might be as effective as transfusion in preventing recurrent stroke and would offer the advantage of avoiding the transfusion-associated problems of alloantibody formation, iron overload, and blood-borne infection risk.

Strengths/Weaknesses: Strengths are the use of a homozygous sickle cell population (except for 1 patient) and evaluation of a novel outcome (stroke). Weaknesses are the descriptive nature of the study, the possible confounding by phlebotomy, the lack of statistical analysis of stroke recurrence, and the lack of reporting of hematologic endpoints other than hemoglobin and hemoglobin F.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation because of the lack of toxicity endpoints.

Ware et al. (2004), support not indicated, published a study on the use of hydroxyurea to prevent stroke recurrence in 35 children, ages 3–19.9 years, with sickle cell disease. The study was designed to correct what the authors believed was a limitation of their previous study (Ware et al., 1999) on prevention of recurrent stroke. In that study, three children had recurrent strokes 3–4 months after abruptly discontinuing prophylactic transfusions. Hydroxyurea, which had been started 2 weeks after discontinuation of transfusions, would not have reached maximum effectiveness for at least 6 months,

according to the authors. In the current study, hydroxyurea was started at 15–20 mg/kg bw/day and was increased by 5 mg/kg bw/day every 8 weeks to a maximum of 30–35 mg/kg bw/day, unless limited by toxicity. Transfusions were continued until hydroxyurea therapy had been increased to the maximum tolerated dose. The mean hydroxyurea dose was 26.7 ± 4.8 mg/kg bw/day **[SD assumed]** (range = 17.0–34.8 mg/kg bw/day). The overlap period during which patients received transfusions and hydroxyurea was 3–15 months, and the duration of hydroxyurea therapy at the time of the report was 3–104 months. As in the previous study, phlebotomy was used during hydroxyurea therapy to decrease iron overload. Seven children had recurrent neurologic events, and four of these seven events occurred within 4 months of starting hydroxyurea. Four of the patients with stroke were considered non-compliant with hydroxyurea therapy. **[Additional information in this paper concerns the effects of phlebotomy on iron status, not discussed here.]** The authors concluded that hydroxyurea is an effective alternative to transfusion for prevention of recurrent stroke in children with sickle cell disease.

Strengths/Weaknesses: Strengths are the prospective design of the study and the larger sample size than the previous study. Phlebotomy remains a possible confounder, and hematologic or other non-neurologic side effects were not reported.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation based on the lack of toxicity endpoints.

Kinney et al. (1999), supported by the NHLBI, presented results of the Pediatric Hydroxyurea Safety Trial (HUGS-KIDS), a Phase I/II multi-center trial of hydroxyurea in 84 children with severe sickle cell anemia. Subjects were 5–15 years old at enrollment (mean ± SD: 9.2 ± 3.2 years) and had been hospitalized at least three times in the previous year for pain events or episodes of acute chest syndrome. The stated aims of the study were to determine: whether hydroxyurea therapy increased fetal hemoglobin levels, hemoglobin concentration, and mean corpuscular volume above baseline values; whether hematologic and other toxicities in children were similar to those in adults; and whether there were adverse effects of hydroxyurea therapy on growth. The initial hydroxyurea dose of 15 mg/kg bw/day was increased by 5 mg/kg bw/day every 8 weeks to a maximum of 30 mg/kg bw/day unless there was drug toxicity. In the face of hematologic toxicity, hydroxyurea was stopped for at least 1 week and was restarted at 2.5 mg/kg bw/day less than the dose at which toxicity occurred. The study was designed to follow subjects for 1 year at the maximum tolerated dose. **[Data tables show 24-month data for 35 subjects, and the authors indicate that the mean ± SD time to attain the maximum tolerated dose was 330 ± 164 days.]** Student's *t*-tests were used to compare hematologic values in the children with values in adults from a different study **[not discussed here]**.

Toxicity indicated by laboratory values included neutropenia in 56 subjects, reticulocytopenia in 35 subjects, anemia in 27 subjects, and thrombocytopenia in seven subjects. Mean changes in laboratory values at 6 months are summarized in Table 26. There was little change in values after 6 months, with the exception of hemoglobin F, which showed further increase at 12 months. Adverse sickle-cell related events included vaso-occlusive crisis in 34 subjects; acute chest syndrome in eight subjects; and gallstones, priapism, splenic sequestration, and transient ischemic attack in one subject each. Events considered not to be related to sickle cell disease included other pain in 29 subjects, nausea/vomiting in 17 subjects, infection in 20 subjects, headaches in 12 subjects, diarrhea in six subjects, rash in five subjects, and bleeding in one subject. None of the children experienced growth failure, which was defined as a growth velocity below the 5th percentile for age over a 6-month period. Height and weight averaged over all children in the study are shown in Figure 5. The authors stated that four girls, ages 11.1–14.1 years, reached menarche while receiving hydroxyurea. **[No assessment of pubertal progression was reported.]**

Table 26
Changes in Laboratory Values After 6 Months of Hydroxyurea in the HUG-KIDS Study

Laboratory measure	Change from enrollment
Hemoglobin	↑13%
Mean corpuscular volume	↑16%
Mean corpuscular hemoglobin	↑16%
Mean corpuscular hemoglobin concentration	↔
Reticulocyte count	↓42%
Leukocyte count	↓32%
Neutrophil count	↓37%
Platelet count	↓20%
Total bilirubin	↓19%
Lactate dehydrogenase	↓18%
Alanine aminotransferase	↔
Creatinine	↔
Hemoglobin F	↑2-fold
Hemoglobin F-containing cells	↑71%

From Kinney et al. (1999).
↑,↓,↔, Statistically significant increase, decrease, or no change, respectively.

The authors concluded that the stability of laboratory measures after the first 6 months of therapy suggested that marrow exhaustion did not occur on continued therapy. They also suggested that frequent adjustments of the hydroxyurea dose to reach the threshold of hematologic toxicity may not be necessary. They stated that hydroxyurea at a daily oral dose of 25–30 mg/kg bw is well tolerated by most children and that 1–2 years of hydroxyurea treatment is "relatively safe."

Strengths/Weaknesses: Strengths are the large sample size, the multi-center design, the use of predefined criteria for growth failure and for hematologic toxicity, and the comprehensive laboratory testing for other causes of anemia and neutropenia. Weaknesses are the 1-year follow-up period and the lack of reported assessment of pubertal progression.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Wang et al. (2001), supported by the American Lebanese-Syrian Associated Charities and NIH, reported results of the Hydroxyurea Safety and Organ Toxicity (HUSOFT) trial, a multi-center study of 28 children, 6–28 months old, treated with hydroxyurea 20 mg/kg bw/

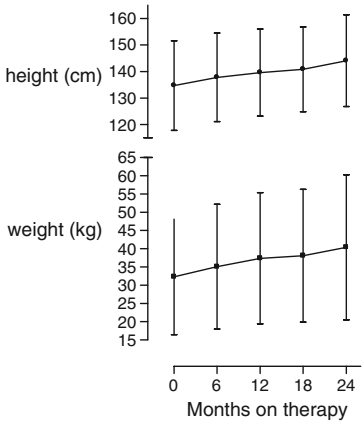

Fig. 5. Growth of children in the HUG-KIDS Study. Data are mean ± SD from Kinney et al. (1999). The number of children at each time point is: entry, 84; 6 months, 78; 12 months, 76; 18 months, 71; 24 months, 35.

day. Doses were adjusted based on laboratory evidence of hematologic toxicity. Subjects were enrolled based on hemoglobin SS (n = 27) or S/β-thalassemia (n = 1) status, without regard to disease severity. Doses were adjusted for weight every 8 weeks, and the planned duration of therapy was 2 years. Laboratory testing was carried out every 2–4 weeks. Technetium 99 m liver-spleen scans were carried out on entry to the study and at study end to assess spleen function. Sixteen patients had magnetic resonance imaging and magnetic resonance angiography of the brain at study entry and end. Neurodevelopmental assessment was carried out at study entry with the Bayley Scales of Infant Development and at study end with the Bayley Scales or the McCarthy Scales of Children's Abilities. Pulse oximetry was carried out quarterly as a surrogate for pulmonary function. Height and weight were assessed every 6 months and compared to historic controls in each center. Comparisons of hematologic data and growth endpoints to same-sex and similar-age children from a previous study (published in 1994 were made using the Wilcoxon/Mann-Whitney test, the Wilcoxon signed rank test, and a quadratic regression model.

The 2-year treatment was completed by 21 subjects. Two patients were discontinued for non-compliance, three were discontinued by their parents for unstated reasons, one patient was placed on a transfusion regimen after a stroke, and one patient died of splenic sequestration syndrome. Most hydroxyurea toxicity was hematologic. Neutropenia ($<1.5\times10^9$/L) occurred in 17 patients, anemia (≥20% decline) in seven patients, and thrombocytopenia ($<80\times10^9$/L) in one patient. Laboratory results are summarized in Table 27. Based on a comparison of liver-spleen scan results from study entry and study end, there was no change in splenic function in 11 subjects, increased function in one subject, and decreased function in five subjects. Two of the 16 children who had brain imaging had evidence of small infarcts; angiography was normal in all 16 cases. The mean developmental scores at study entry (93.7) and study end (89.5) were not significantly different. Pulse oximetry showed >95% oxyhemoglobin saturation without significant change over the course of the study. Growth velocity was normal during the study and did not differ from age-matched retrospective control values. Head circumference percentiles did not change over the course of the study. There were two patients with splenic sequestration, one of whom died. One patient was diagnosed with stroke (mentioned above as having been discontinued from the study on that basis), and one patient had a transient ischemic attack.

The authors concluded that hydroxyurea therapy in very young children is feasible and well-tolerated and that hematologic toxicity is limited and manageable.

Strengths/Weaknesses: Strengths are the relatively large sample size, the use of the same maximum hydroxyurea dose in all children, the homozygosity of the patients (with 1 exception), the evaluation of young children, the use of predefined criteria for hematologic toxicity, comparison with genotype-matched historic controls, and neurodevelopmental assessment using age-appropriate validated scales. Weaknesses are the relatively low hydroxyurea dose, enrollment regardless of severity, and follow-up for only 2 years, which may not be sufficient to show growth effects. In addition, pulse oximetry is not the best measure of pulmonary function in patients with sickle cell disease.

Table 27
Hematologic Changes in Very Young Children on Hydroxyurea for 2 Years

Laboratory test	Compared to entry[a]	Compared to 1994 study
Hemoglobin	↔	↑14%
Mean corpuscular volume	↑10%	↑7%
Percent hemoglobin F	↔	↑1.9-fold
Hemoglobin F containing cells	↔	↑17%
Leukocyte count	↓20%	↓29%
Platelet count	↔	↔

From Wang et al. (2001).
[a]*t*-test performed by CERHR.
↑,↓,↔, Statistically significantly increase, decrease, or no change, respectively.

Table 28
T-cell Receptor Translocation Events Associated with Hydroxyurea Treatment

Group (*n*)	Events per μg DNA
Children not on hydroxyurea (21)	1.06 ± 0.45
Children on hydroxyurea for a median of 7 months (17)	1.58 ± 0.87[a]
Children on hydroxyurea for a median of 30 months (17)	1.82 ± 1.20[b]

From Hanft et al. (2000).
[a]$p = 0.023$ compared to children not on hydroxyurea, *t*-test by CERHR assuming the data represent mean ± SD.
[b]$p = 0.011$ compared to children not on hydroxyurea, *t*-test by CERHR assuming the data represent mean ± SD. **[The Expert Panel notes that this group includes the same children evaluated at 7 months,** and **that the *t*-test is not ideal under those circumstances. Data were not available for a repeated-measures test.]**

Appendix II

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Hanft et al. (2000), supported by the Duke Children's Miracle Network Telethon and the Leukemia Society of America, evaluated acquired mutations associated with exposure to hydroxyurea in 17 children with sickle cell disease. **[Adults were also evaluated, and that part of the study was discussed in Section 2.4.]** A group of 21 children with sickle cell disease who were not treated with hydroxyurea was also evaluated. At the initial evaluation, the children in both groups were 11 ± 3 years old and the hydroxyurea-treated children had been on therapy for a median of 7 months. These children were re-evaluated after a median of 30 months of exposure. Mononuclear cells were isolated from peripheral venous blood and used in the *HPRT* assay and T cell receptor interlocus recombination events (at the Vγ and Jβ loci). Group comparisons were made using the Student's *t*-test. Comparisons at different time points were made using the Wilcoxon signed-rank sum test. **[Despite this statement, the Results section indicates that the "3 groups," children without HU exposure, children with HU exposure for a median of 7 months, and the same children with HU exposure for a median of 30 months, were evaluated using ANOVA.]** Hydroxyurea treatment was not associated with a statistically significant change in *HPRT* mutation frequency. Children on hydroxyurea had more Vγ-Jβ translocation events than children who were not on hydroxyurea (Table 28). The authors concluded that the mutagenic and carcinogenic potential of hydroxyurea was low, but that "[l]ong-term serial measurements of acquired…mutations in young patients with sickle cell disease on hydroxyurea therapy may be warranted."

Strengths/Weaknesses: Strengths are the long duration of exposure and the reliability of the assays used to assess mutation. The failure to use a repeated measures test when the same children assessed at 7 months were reassessed at 30 months is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is one of only two studies on mutation associated with hydroxyurea treatment of children with sickle cell disease, and it is important to consider the study in the evaluation process.

Schultz and Ware (2003), support not indicated, published results of a survey of members of the International Association of Sickle Cell Nurses and Physician Assistants. Members were asked to identify all patients with sickle cell disease known to have cancer. There were 49 patients reported to have one or more cancer diagnoses; 21 of the cancer patients were children. One of the children was reported to have received hydroxyurea 3 months before the diagnosis of acute lymphocytic leukemia. Two adult cancer patients also had been exposed to hydroxyurea. **[There was no estimate of the proportion of the underlying sickle cell disease population in which hydroxyurea had been used.]** The authors did not draw conclusions relevant to hydroxyurea.

Strengths/Weaknesses: The strength of this survey is the large number of affected patients who were identified, giving some idea of cancer prevalence in sickle cell disease patients. Weaknesses include the reliance on reporting, with the potential for inaccurate or incomplete information, and the lack of estimate of the proportion of the sickle cell disease population in which hydroxyurea was used.

Utility (Adequacy) for CERHR Evaluation Process: The Expert Panel has little confidence in the accuracy of calculations based on the information in this study. The study is not useful for the evaluation.

Hankins et al. (2005), supported by the American Lebanese-Syrian Associated Charities and NIH, reported an extension of the HUSOFT study, which was the subject of a previous report (Wang et al., 2001). The original trial was designed to evaluate the effects of hydroxyurea on 6–24-month-old children with sickle cell disease, particularly with respect to its safety and its ability to prevent organ damage. Children in that study had been given hydroxyurea 20 mg/kg bw/day. At the end of the 2-year trial, guardians of all 21 subjects who were still in the study agreed to participate in the continuation study. Hydroxyurea was increased by 5 mg/kg bw/day every 6 months to 30 mg/kg bw/day. Hematology and serum chemistry testing was carried out every 6 months, and liver-spleen scan was carried out at the end of 2 and 4 years. Magnetic resonance imaging and magnetic resonance angiography were carried out every 2 years. Comparisons were made between the hematologic data in this study and data published in 1994 from children with sickle cell disease who were followed prospectively without treatment. Independent *t*-test was used for data distributed normally, and the Wilcoxon ranked-sum test was used for other data.

Of 21 subjects who started the extension study, 17 completed an additional 4 years, and 11 were treated for an additional 6 years. Compared to the end of Year 2, when the extension study began, there were no additional changes in hematologic endpoints, with the exception of hemoglobin F, which increased an additional 17%. **[The Year 2 data are discussed in the summary of Wang et al. (2001) and are shown in Table 27.]** Neutropenia occurred in association with viral illness in some of the children. Anemia attributed to aplastic crises also occurred. **[It is not possible to tell how many children were affected.]** The one death in the extension study was attributed to sepsis. Of 14 patients who had not undergone splenectomy by the start of the extension study and who had liver-spleen scans after 2 and 4 years on hydroxyurea, three had normal splenic uptake, five had decreased update, and six had no uptake and were considered functionally asplenic. Of 14 patients who had brain imaging after 4 years of hydroxyurea therapy, three had evidence of infarctions. There was an average yearly increase of 2.15 kg in weight, 7.9 cm in height, and 1.7 cm in head circumference, with normal growth rates using standardized curves. Boys increased their weight percentile from 25 to 50 and their height percentile from 40 to 50 after 4 years of therapy. Height and weight were higher in hydroxyurea-treated children than in untreated children with sickle cell disease in the 1994 publication

The authors concluded that hydroxyurea is a "relatively safe drug" for children with sickle cell disease but its use requires periodic monitoring of blood counts and physical examinations. They characterized the possibility of myelodysplasia or malignancy as an ongoing concern.

Strengths/Weaknesses: The continuation of the previous study is a strength. Other strengths are the prospective nature of the study, the increase in

hydroxyurea to a single fixed dose, the long-term follow-up of some of the patients, and the standardized outcome measures of splenic function, magnetic resonance imaging, and growth endpoints. The small number of patients, particularly at the longer follow-up intervals, is a weakness. The association of neutropenia with viral illness in some patients does not constitute toxicity of the medication.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Fung et al. (2001), supported by the NHLBI and the Children's Hospital of Philadelphia, evaluated resting energy expenditure in eight children, ages 5.2–9.6 years, on hydroxyurea for sickle cell disease. The children were enrolled in the HUG-KIDS study, which had been reported previously (Kinney et al., 1999). The initial hydroxyurea dose in that study was 15 mg/kg bw/day, with an increase by 5 mg/kg bw/day every 8 weeks unless toxicity occurred. The children in this sub study were evaluated before therapy and after a hydroxyurea dose of 20 mg/kg bw/day was tolerated. The duration of treatment before re-evaluation was 4.1–14.5 months. Height, weight, and triceps skinfold thickness were measured. Skinfold thickness was used to estimate fat mass, percent body fat, and fat-free mass. Resting energy expenditure was measured for 60 min using open-circuit indirect calorimetry. **[This technique involves spirometry with measurement of oxygen consumed and carbon dioxide produced.]** Dietary intake was estimated using 3-day recordings of food consumption. Statistical analysis used paired *t*-tests to compare baseline and on-therapy values and to determine whether *z*-scores for height and weight were different from zero. Longitudinal mixed effect-analysis was used to evaluate changes in resting energy expenditure over time, with adjustment for fat-free mass, sex, energy intake, disease severity, hemoglobin, and hemoglobin F levels.

Based on an evaluation of *z*-scores, the children in this study were taller and lighter and had lower skinfold measurements than healthy children. All subjects gained height, weight, and skinfold thickness on therapy, as expected for growing children, with an improvement of the weight-for-height *z*-score so that it no longer differed from the reference population of healthy children. Although parents reported improved appetite on hydroxyurea therapy, energy intake as a percentage of recommended energy intake did not change on therapy. Resting energy expenditure did not change on therapy when analyzed in kilocalories (kcal)/day. When analyzed as a percentage of the World Health Organization predicted values (which are age- and sex-specific), resting energy expenditure decreased by an average of 8%. Six of the subjects had elevated resting energy expenditures at baseline, and hydroxyurea therapy was associated with a reduction to normal energy expenditure levels. The remaining two subjects had normal resting energy expenditures at baseline that remained normal. There were significant effects of fat-free mass and hemoglobin F level on resting energy expenditure, with a 14.4 kcal/day decrease in resting energy expenditure associated with each 1% increase in hemoglobin F.

The authors concluded that elevated resting energy expenditure is a likely contributor to the undernutrition and growth failure associated with sickle cell disease. The authors postulated that a decrease in red blood cell destruction on hydroxyurea would decrease the metabolic energy needs for new cell production and that the decrease in sickling events would decrease inflammation with its metabolic energy requirements.

Strengths/Weaknesses: The homogeneity of the population is a strength, as is the novel effect, not studied previously. Weaknesses are the small sample size and the short follow-up period, which was not adequate for an assessment of growth.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility for the evaluation.

Altura et al. (2002), supported by NIH and the American Lebanese Associated Charities, evaluated serum magnesium and other cations in five girls, ages 11–14, who were part of the HUG-KIDS study, reported previously (Kinney et al., 1999). The subjects were treated with hydroxyurea 15 mg/kg bw/day with planned increases to a maximum of 30 mg/kg bw/day. The mean dose at the end of 18 months was 27 mg/kg bw/day (range, 20–30 mg/kg bw/day). Serum ionized magnesium, calcium, sodium, and potassium were assessed at baseline and every 6 months. Statistical comparisons were made to ''healthy control'' values **[source not indicated]** and baseline values by Student's *t*-test and ANOVA with post-hoc Dunnett or Scheffé test. Before hydroxyurea, mean $\pm$ SEM ionized magnesium was 0.53 ± 0.03 nM (normal range = 0.51–0.67). On therapy, ionized magnesium decreased further to 0.47 ± 0.03. Mean total magnesium also declined with therapy. The other cations remained in the normal range. The authors concluded that patients with sickle cell disease have low serum magnesium, which may play a role in erythrocyte hydration and vascular reactivity, and that hydroxyurea therapy exacerbates this magnesium deficiency. They proposed a trial of supplemental magnesium as part of sickle cell disease treatment.

Strengths/Weaknesses: A strength of this study is the demonstration of a novel type of hydroxyurea toxicity. A weakness is the small sample size.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Wang et al. (2002), support not indicated, reported on growth in 68 children treated with hydroxyurea for sickle cell disease. The children were part of the HUGS-KIDS study, which was previously reported (Kinney et al., 1999), and were selected for having reached the maximum tolerated dose in that study **[30 mg/kg bw/day according to Kinney et al. (1999)]**. The children were 5–16 years old at study entry and had records of at least 2 years of pre-study height and weight measurements. Hydroxyurea was increased to the maximum tolerated dose, after which height and weight were measured in the children for 1 year. Serial assessments of Tanner stages were also recorded. Age-adjusted growth data of children in the HUGS-KIDS study were compared to data from a report published previously of a group of children with sickle cell disease not treated with hydroxyurea. Height and weight for age were similar in HUGS-KIDS subjects before hydroxyurea therapy and while on therapy. Height (beginning at age 7) and weight (beginning at age 9) were greater in HUGS-KIDS subjects than in the historic population of untreated children with sickle cell disease, which the authors indicated may have been due to the 16-year difference between the studies. Height and weight velocity measurements were highly

variable, and differences between the groups could only be shown for three individual year-sex subgroups. Thirty-five children remained at Tanner Stage 1 throughout the study, but 21 of the children were <10 years old at study end and would not have been expected to be beyond Tanner Stage 1. Pubertal progression was assessed by calculating the age at which there was a 50% likelihood of each Tanner stage transition. For girls, these ages were 11.4 years for the Tanner 1–2 transition, 13.8 years for the Tanner 2–3 transition, and 15.3 years for the Tanner 3–4 transition. For boys, these ages were 12.1 years for Tanner 1–2, 14.4 years for Tanner 2–3, and 15.8 years for Tanner 3–4. The authors indicated that these ages were comparable to those reported in the historic comparison group. The authors concluded that hydroxyurea treatment had no adverse effect on growth or pubertal development.

Strengths/Weaknesses: Strengths are a good historic control group, inclusion of measurements for 2 years before therapy, strict inclusion criteria, the prospective design, good sample size, and defined criteria for pubertal progression. The short follow-up duration is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Wang et al. (2005), supported by the American Lebanese-Syrian Associated Charities, analyzed height and weight data from a study of long-term transfusion in children with sickle cell disease. The children had been randomized to regular transfusion or to standard care after it was determined by transcranial Doppler velocimetry that they were at high risk of stroke. In the current study, the authors compared 2-year growth data from the intervention group to data from the standard care group **[not discussed here]**. They also compared height and weight velocity in the children in this trial with those in the HUG-KIDS study published previously (Wang et al., 2002), in which hydroxyurea was given to children for the treatment of sickle cell disease. Height and weight velocity were expressed in cm/month and kg/month, respectively, and were derived by combining data from all 5–16-year-old children in each study. No significant differences were found between height and weight velocity in hydroxyurea-treated children and in children from either the control or intervention groups in the transfusion study. The authors' conclusions were confined to comments about transfusion therapy.

Strengths/Weaknesses: The primary purpose of this study was to compare chronic transfusion therapy to standard care, and children on hydroxyurea were used only as a comparison group for evaluation of growth. The lack of information on possible hydroxyurea toxicity is a weakness for the purposes of this evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation.

Zimmerman et al. (2004), support not indicated, reported outcome data from Duke University on 122 children with sickle cell disease who were treated with hydroxyurea according to different protocols. Some of the children were reported previously in the HUG-KIDS study (Kinney et al., 1999), and some were reported as part of a trial of hydroxyurea in very young children (Wang et al., 2001). The age range of the children at initiation of therapy was 0.5–19.7 years, and the duration of therapy ranged from 6–101 months. Hydroxyurea was started at 15 or 20 mg/kg bw/day and was increased at different rates to the maximum tolerated dose or to 30 mg/kg bw/day. In some children, hydroxyurea was given at 35 mg/kg bw/day. The mean ± SD hydroxyurea dose was 25.4 ± 5.4 mg/kg bw/day. Laboratory criteria for stopping hydroxyurea temporarily varied by protocol. Laboratory data were abstracted from clinical records. Height and weight data were obtained annually. To assess mutagenicity, 34 patients on therapy for at least 5 years were evaluated for gene rearrangements between T cell receptor loci on chromosome 7 (Vγ-Jβ recombination events). Comparisons of laboratory values at baseline and at the maximum tolerated dose of hydroxyurea were made with Student's *t*-test.

Table 29
Laboratory Values in Children on Maximum Tolerated Doses of Hydroxyurea

Laboratory test	Change from baseline
Hemoglobin	↑18%
Mean corpuscular hemoglobin	↑25%
Percent hemoglobin F	↑2.5-fold
Reticulocyte count	↓48%
Leukocyte count	↓44%
Neutrophil count	↓44%
Platelet count	↓22%
Bilirubin	↓39%

From Zimmerman et al. (2004).
↑,↓, Statistically significant increase or decrease, respectively.

Table 30
Hematologic Values on Maximum Dose of Hydroxyurea in Children Ages 2–5

Laboratory test	Comparison to baseline value
Percent hemoglobin F	↑2.9-fold
Hemoglobin	↑26%
Mean corpuscular volume	↑26%
Leukocyte count	↔
Neutrophil count	↓38%
Platelet count	↔

From Hoppe et al. (2000).
↑,↓,↔, Statistically significant increase, decrease, or no change, respectively.

The changes in laboratory values are summarized in Table 29. After the first year of therapy at the maximum tolerated dose, there were no significant additional changes in the laboratory measures. The principal toxicity in this group of patients was hematologic, manifested as abnormal laboratory values that responded to temporary cessation of therapy. Mild nail and skin changes occurred in 10% of patients but did not lead to discontinuation of therapy. Gastrointestinal irritation was described in an unspecified number of patients. The authors stated that serial measures of height and weight showed no adverse effects of hydroxyurea therapy. **[The data were summarized in figures that gave averages for the entire sample without adjustment for age.]** Recombination events were not increased compared to baseline in children who had been on hydroxyurea for at least 5 years. There were no cases

Table 31
Laboratory Results After 12 Months of Hydroxyurea in 6 Children with Sickle Cell Disease

Laboratory test	Comparison to baseline
Hemoglobin	↔
Mean corpuscular volume	↑33%
Leukocyte count	↓31%
Neutrophil count	↓44%
Platelet count	↔
Reticulocyte count	↔
Total bilirubin	↔
Alanine aminotransferase	↔
Aspartate aminotransferase	↔
Creatinine	↔
Percent fetal hemoglobin	↑5.5-fold
Percent hemoglobin F-containing cells	↑3.3-fold

From Miller et al. (2001).
↑,↓,↔, Statistically significant increase, decrease, or no change compared to baseline.

of myelodysplasia, leukemia, or other malignancy in these children. None developed serious infection associated with leukopenia, although one girl died of pneumococcal bacteremia despite having a normal leukocyte count before infection. Another child died of a transfusion reaction. The authors concluded that based on its effectiveness **[not discussed here]** and low toxicity, hydroxyurea can be considered for use in all patients with sickle cell disease.

Strengths/Weaknesses: Strengths are the large sample size, the analysis of results by sickle cell genotype, the attempted assessment of compliance with hydroxyurea therapy, and the long-term follow-up in some of the children. Weaknesses are the varied hydroxyurea doses and laboratory criteria for cessation of therapy, the lack of documentation of pubertal assessments, the lack of reporting of growth velocities, and the failure to adjust the statistical analyses for multiple comparisons. The reliability of the growth assessments is seriously compromised by reporting averages for the sample without adjustment for age. The inclusion of the results from two other studies is another weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Hoppe et al. (2000), supported by NIH, reported eight children, ages 2–5 years, treated with hydroxyurea for sickle cell disease. The treatment was started at 15 mg/kg bw/day and increased by 5 mg/kg bw/day every 8 weeks to 30 mg/kg bw/day unless toxicity limited the dose increases. Patients were followed with complete blood count, serum chemistries, and hemoglobin F measurements. Height and weight were measured serially. Hematologic results and clinical events on therapy were compared to those recorded before therapy using the Wilcoxon signed-rank test. The treatment period was 56–290 weeks **[1–5.6 years]**. Hematologic results obtained from patients at their maximum tolerated hydroxyurea doses are summarized in Table 30. There were reductions in hospital admissions, days in the hospital, and blood transfusions. There were no deviations in individual growth percentiles, and developmental milestones were attained at appropriate ages. One patient had a stroke after 1 year of therapy, although hematologic measures and clinical course to that point suggested a good response to hydroxyurea. The authors concluded that young children responded to hydroxyurea therapy for sickle cell disease similarly to older children and adults. They concluded that there were no detectable hydroxyurea effects on growth but cautioned that standard growth curves and developmental milestones were relatively gross measures and that more formal assessment of growth and development on hydroxyurea would be desirable.

Strengths/Weaknesses: Strengths are the use of strict inclusion criteria, the inclusion of only homozygous sickle cell patients, the inclusion of young children, and the 3-year follow-up period. The use of the Denver Developmental Screening Test is a strength in that the test is standardized but a weakness in that only gross developmental milestones were assessed. Other weaknesses are the small sample size and the lack of specification on the assessment frequency for growth endpoints. Few patients had myelosuppression, raising the question of compliance with the hydroxyurea regimen.

Utility (Adequacy) for CERHR Evaluation Process: This study has utility for the evaluation process.

Miller et al. (2001), support not indicated, treated six children, ages 6.7–17.5, with hydroxyurea for hemoglobin SC disease. Treatment was started at 15 mg/kg bw/day and increased by 5 mg/kg bw/day every 8–12 weeks to 30 mg/kg bw/day unless limited by toxicity. Hematology and blood chemistry determinations at 12 months of therapy were compared to baseline values using the Wilcoxon signed-rank test (Table 31). The duration of therapy was 12–41 months at the time of the report. Three patients required temporary cessation of therapy due to hematologic toxicity, and neutropenia or thrombocytopenia prevented any patient from reaching the target dose. All patients experienced a decrease in the number and severity of vaso-occlusive events and a decrease in hospitalization. The authors concluded, based on what they described as a pilot trial in adults, that children with SC disease had a larger hydroxyurea-mediated increase in fetal hemoglobin than did adults. They postulated that adults might have more interindividual variability, could be less responsive or less compliant, or may have received suboptimal doses of hydroxyurea. They proposed alternatively that children were more responsive because the γ-globin gene had been silenced for less time in children than in adults.

Strengths/Weaknesses: Strengths are the use of up to 40 mg/kg bw/day hydroxyurea and the predefined hematologic criteria for stopping therapy. The small sample size is a weakness. The study of children with SC disease is a strength in its focus on this subpopulation and a weakness in its limitation on generalizability to other sickle cell disease genotypes.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Moschovi et al. (2001), support not indicated, presented a case report of an 8-year-old boy who was diagnosed with Stage II lymphocyte-predominant Hodgkin's disease 6 months after starting hydroxyurea therapy for sickle cell disease. The hydroxyurea therapy appeared to have improved the child's clinical condition before the diagnosis of the Hodgkin's disease. The dose of hydroxyurea was 20 mg/kg bw/day. The hydroxyurea

was discontinued after the diagnosis, and therapy with adriamycin, bleomycin, vinblastine, and dacarbazine was given for the Hodgkin's disease. The patient continued to be in remission at the time of the report, which was about 5 years after the diagnosis. The sickle cell disease was managed successfully with bone marrow transplantation. The authors concluded, based on the short duration of hydroxyurea therapy, that there was no relationship between hydroxyurea and Hodgkin's disease in this child, but they questioned whether there might be an interaction between Ebstein-Barr virus and hydroxyurea in the development of this malignancy.

Strengths/Weaknesses: Although this study is interesting, the single case is a weakness for the purposes of this evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation.

O'Branski et al. (2001), supported by the NHLBI, presented a case series of seven children with skin and nail changes attributed to hydroxyurea therapy for sickle cell anemia. The children ranged in age from 9–18 years, and their hydroxyurea doses were 17–25 mg/kg bw/day. The duration of therapy before the integument changes was 6–16 weeks. The most common change was longitudinal banding of pigment in the nails, affecting 6 of the 7 children. Four children had nail hyperpigmentation, three children had pigmentation of the palmar creases, and one child had hyperpigmented macules on the chest and back. The authors estimated, based on the number of hydroxyurea-children at their center, that the incidence of nail and skin changes was about 15%.

Strengths/Weaknesses: The strength of this study is the attention to an outcome that has only been noted intermittently in other studies. Weaknesses are the small sample size, the possible retrospective chart-review nature of the data retrieval, and the lack of clarity on whether all children treated with hydroxyurea were assessed for nail and skin changes.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Sumoza et al. (2002), support not indicated, reported on five children with sickle cell disease and a history of stroke who were placed on hydroxyurea to prevent recurrent stroke. The children were 3, 8, 10, 10, and 16 years old. Hydroxyurea was given at 30 mg/kg bw/day in one patient and 40 mg/kg bw/day in the others with 42–112 months of follow-up. None of the children had a recurrent stroke while taking hydroxyurea. One subject was on hydroxyurea discontinuously and suffered a transient ischemic attack and a stroke during the two periods of time that she had discontinued therapy. No painful crises occurred during hydroxyurea therapy. Total hemoglobin and hemoglobin F increased on therapy. There were no episodes of recognized leukopenia or thrombocytopenia. The authors concluded that their results were promising but that a larger study with long-term follow-up was needed.

Strengths/Weaknesses: Strengths are the inclusion of only homozygous hemoglobin SS patients, the use of a maximum tolerated dose of 30–40 mg/kg bw/day, and long-term follow-up. The focus on children with a stroke history is also a strength. The small sample size is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Table 32
Laboratory Results after 12 Months of Hydroxyurea Treatment in Saudi Patients

Laboratory value	Comparison to baseline
Leukocyte count	↓30%
Hemoglobin	↑11%
Mean corpuscular volume	↑19%
Hemoglobin F	↑2-fold
Platelet count	↓36%
Reticulocyte count	↓39%
Lactate dehydrogenase	↓29%
Total bilirubin	↔

From Al-Jam'a and Al-Dabbous (2002).
↓,↑,↔, Statistically significant decrease, increase, or no change compared to baseline, respectively.

Al-Jam'a and Al-Dabbous (2002), support not indicated, reported the use of hydroxyurea in 36 Saudi Arabian patients with sickle cell disease. The patients were 10–36 years old. **[Results were not given by age or by adult/child status.]** The starting dose of hydroxyurea was 500 mg/day for subjects weighing >50 kg and 500 mg every other day for subjects weighing <50 kg (about 8–10 mg/kg bw/day), and doses were increased as tolerated to 35 mg/kg bw/day. Hematology and clinical chemistry results were followed, and patients recorded symptoms in a diary. Twenty-seven patients who completed 12 months of therapy were the subject of this report. Laboratory results are summarized in Table 32. Although 20 subjects had at least a 2-fold increase in hemoglobin F, there was considerable variability, with a range of 1.3- to 18-fold. There was a decrease in number of painful episodes, number of hospital admissions, and number of days/year in the hospital. The authors concluded that hydroxyurea was effective in decreasing the frequency of vaso-occlusive crises but that long-term follow-up was needed.

Strengths/Weaknesses: Strengths include the evaluation of sicker patients (4 vaso-occlusive crises in the previous year) and predefined criteria for myelotoxicity. Weaknesses are the open-label, uncontrolled design, the use of different dose schedules, and the failure to report results by age.

Utility (Adequacy) for CERHR Evaluation Process: This study may have some utility because the resists are consistent with the hematologic findings in other studies; however, the failure to distinguish results in children from those in adults is a serious limitation.

Koç et al. (2003), support not indicated, reported on 11 children, 8–18 years old, who were treated with hydroxyurea for sickle cell disease or β-thalassemia intermedia. The hydroxyurea dose was 15–25 mg/kg bw/day. Patients were followed for 5–6 months. Hematology testing was carried out, and levels of clotting factors were determined before and during therapy. Statistical comparisons were made between baseline and on-therapy results using the Wilcoxon signed-rank test. There was a statistically significant 34% decrease in Factor VIII and a 16% decrease in protein C levels. No other statistically significantly alterations in clotting factors were identified. There were increases in hemoglobin, hemoglobin F, percentage of cells containing hemoglobin F, mean

corpuscular volume, and mean corpuscular hemoglobin. **[Data were not shown.]** The authors concluded that a hydroxyurea-mediated decrease in Factor VIII may decrease the hypercoagulability of sickle cell disease and protect against vaso-occlusive crisis.

Strengths/Weaknesses: The focus on the coagulation system, which had not been studied previously in this patient group, is a strength. Weaknesses are the small sample size, short follow-up, and lack of data on other hematologic endpoints.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Bakanay et al. (2005), supported by the NHLBI, evaluated mortality among patients with sickle cell disease who had ever received hydroxyurea. There were 226 patients treated with hydroxyurea at the authors' center. The patients were 16–68 years old. Thirty-eight of the patients were dead at the time of the study, and hydroxyurea was being used by 26 of them at the time of death. Comparisons were made between deceased patients and survivors based on retrospective analysis. The mean $\pm$ SD age of onset of hydroxyurea therapy was higher in deceased patients (30.6 $\pm$ 11.3 years) than in surviving patients (26.4 $\pm$ 9.5 years). **[Laboratory comparisons of deceased and surviving patients are not discussed here.]** The authors concluded that institution of hydroxyurea therapy at younger ages should be considered.

Strengths/Weaknesses: The large sample is a strength of this study. Weaknesses are the retrospective data collection, the potential confounders in comparing deceased and living patients, and the lack of a focus on children. The authors' conclusions are not necessarily supported by their data.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation.

Braga et al. (2005), support not indicated, reported the experience of nine children given hydroxyurea for sickle cell disease in Portugal. Two children were subsequently excluded for non-compliance. The remaining seven children ranged in age from 9–15 years. Two of these children received transfusions in addition to hydroxyurea. The initial hydroxyurea dose was 15 mg/kg bw/day with increases as tolerated to 25 mg/kg bw/day. Hematology and blood chemistry measurements were taken before hydroxyurea therapy and every 3 months thereafter for 15 months. Baseline and 15-month values were compared statistically using paired *t*-tests. Hemoglobin F increased from a median of 6.6–14.5% in the five children who were not transfused. Mean corpuscular volume and mean corpuscular hemoglobin were described as having increased (mean corpuscular volume by 11%, $P = 0.094$, and mean corpuscular hemoglobin by 14%, $P = 0.097$). Total bilirubin decreased 44% ($P = 0.051$). No significant changes were noted in hemoglobin, reticulocyte count, neutrophil count, platelet count, lactate dehydrogenase, blood urea nitrogen, creatinine, aspartate aminotransferase, or alanine aminotransferase. One child on hydroxyurea developed a leg ulcer. One patient developed neutropenia (neutrophil count $< 2000/mm^3$), which was attributed to an intercurrent viral infection rather than to hydroxyurea. The authors concluded that low toxicity was associated with hydroxyurea therapy of sickle cell disease in children.

Strengths/Weaknesses: Strengths are the use of strict inclusion criteria, the attempt to assess patient compliance by pill count, and the predefined criteria for hematologic and hepatic toxicity. Weaknesses are the small sample size, the use of hydroxyurea doses only up to 25 mg/kg bw/day, and the use of blood transfusion. The homozygous Bantu haplotype in five of seven patients may limit generalizability of the findings.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation.

Elira Dokekias et al. (2005), support not indicated, described the use of hydroxyurea in 132 patients with homozygous sickle cell disease **[presumably sickle cell anemia]** in Tunisia. The patients included five who were younger than 15 years old and 47 who were 15–25 years old. **[The authors describe the youngest child as being 14 years in one section, but in another, give a lower age limit of 12 years. Results were not given by child/adult status.]** The patients were given hydroxyurea 10–30 mg/kg bw/day; 108 of the 132 patients were followed for at least 18 months, and 65 patients were followed for at least 24 months. Blood counts were carried out monthly, and hemoglobin electrophoresis was carried out annually. Statistical methods were not discussed. The authors described a 30% increase in hemoglobin, a 20% increase in mean corpuscular volume, and a 21% decrease in reticulocyte count in patients treated for 1 year. **[Reticulocyte counts were not carried out on all patients due to lack of reagents.]** Patients experienced a reduced number of vaso-occlusive crises, with disappearance of crises in $>80\%$ of patients on hydroxyurea for >12 months.

Strengths/Weaknesses: Strengths are the use of homozygous sickle cell patients and the large sample size. Weaknesses are the failure to analyze results by age, the inclusion of few children, the use of various doses of hydroxyurea, the lack of statistical analysis, and the interruption of the study by a civil war.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation.

Karimi et al. (2005), supported by the Shiraz University of Medical Sciences, reported the use of hydroxyurea in 163 patients with thalassemia intermedia. The patients were 4–35 years old (mean = 13.5 years). **[Children were not distinguished from adults elsewhere in the report.]** The starting hydroxyurea dose was 8–12 mg/kg bw/day. Patients were followed for up to 6 years. Hematology tests were carried out annually, and statistical testing was carried out to compare results between years **[not discussed here]**. Comparisons with baseline values were not made because subjects were being transfused at baseline. Transfusion requirements decreased in the treated subjects, and subjects said they had increased exercise tolerance and more energy. Leukopenia and thrombocytopenia were rare. The authors concluded that hydroxyurea was a well-tolerated treatment for thalassemia intermedia.

Strengths/Weaknesses: Strengths are the large sample size and the long-term follow-up. Weaknesses are the inclusion of only thalassemia patients, which may limit generalizability to sickle cell patients; the different doses of hydroxyurea; and the use of subjective outcome measures.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation but limited by failure to report results by age.

3.1.4 Treatment of childhood malignancies. There are a number of studies with health and reproductive outcomes in survivors of childhood cancer treated with different modalities. Reference to hydroxyurea was made in a description of the design of the Childhood Cancer Survivor Study (Robison et al., 2002). Hydroxyurea was a part of the treatment regimen in 510 (4%) of the 12,455 study participants. Most of the children who received hydroxyurea were treated for leukemia, central nervous system (CNS) tumors, or non-Hodgkin lymphoma. The studies that presented the results of the Childhood Cancer Survivor Study did not further mention hydroxyurea, and it is not possible to associate particular outcomes with hydroxyurea exposure history.

Harrod et al. (2007), support not indicated, reported a technique to measure Howell-Jolly bodies (micronuclei) in erythrocytes as a quantitative assessment of splenic function in children with sickle cell disease. Because the spleen normally filters out circulating erythrocytes with micronuclei, an increase in the number of erythrocytes with Howell-Jolly bodies was believed to represent functional asplenia. Subjects were 147 patients 0–19 years old with hemoglobin SS or SC disease. Blood collected from these children as a part of routine clinical care was placed in fixative, and cells were collected by centrifugation. Young reticulocytes were identified using a fluorescent anti-CD71 antibody, and DNA was identified using propidium iodide. Howell-Jolly body-containing cells were quantified by flow cytometry. Clinical predictors of the proportion of Howell-Jolly body-containing cells were evaluated initially using simple linear regression to identify candidate variables followed by multiple linear regression using a backward elimination procedure. Howell-Jolly bodies were more prevalent in children with hemoglobin SS. The frequency of Howell-Jolly body-containing young reticulocytes and older erythrocytes was increased by hydroxyurea exposure, age, and splenectomy. The authors could not determine whether the association between hydroxyurea exposure and the prevalence of Howell-Jolly bodies was due to hydroxyurea-induced alterations in erythropoiesis kinetics or to drug-induced genotoxicity. **[It also cannot be determined whether the number of Howell-Jolly bodies might also represent underlying disease status.]**

Strengths/Weaknesses: The use of flow cytometry to quantify Howell-Jolly bodies is a nice tool and probably represent a measure of splenic dysfunction. A strengths is the large sample size. It is not clear, however, why the early red cells (reticulocytes) with Howell-Jolly bodies would represent the effect of cytotoxic exposure and the non-reticulocytes with Howell-Jolly bodies would represent functional asplenia. It would be particularly difficult to make this distinction in sickle cell disease where the red cell has such a short half life to begin with. It is a weakness that there were no data on how many Howell-Jolly bodies were seen in the subjects before hydroxyurea exposure. It is possible that the children were placed on hydroxyurea because they were sicker, therefore more likely to have infarcted their spleen, and therefore more likely to have more Howell-Jolly bodies. It is not possible to draw conclusions about the use of this technique as a measure of hydroxyurea toxicity.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation.

3.2 Experimental Animal Data

3.2.1 Rat. Studies in rats are organized by exposure route and according to prenatal or postnatal observations. In each section, studies including multiple doses are presented first, followed by other studies presented in the order that each was published.

3.2.1.1 Oral dosing: Aliverti et al. (1980), support not indicated, examined developmental toxicity in rats exposed to hydroxyurea through the oral or i.p. dose

Table 33
Developmental Toxicity in Rat Offspring Orally Dosed with Hydroxyurea on GD 6–15

	Administered dose (mg/kg bw/day)				Benchmark dose[a] (mg/kg bw/day)			
Endpoint	50	150	300	450	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Postimplantation loss	↔	↔	↑779%	↑1126%	125	114	—	—
Mean fetal weight[b]	↔	↔	↓28%	↓39%	164	119	146	101
Fetuses with malformations[c]								
External	0/123	0/123	4/51	12/30	329	293	—	—
Visceral	0/63	0/63	6/30	15/16	282	248	—	—
Skeletal	0/60	0/58	3/21	11/14	287	243	—	—

From Aliverti et al. (1980).
[a]The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The $BMDL_{10}$ represents the dose associated with the lower 95% confidence interval around this estimate. A 10% alteration in a continuously distributed parameter is an arbitrary benchmark that may not be comparable to a similar alteration in any other endpoint. The BMD_{1SD}, which represents an alteration equivalent to 1 SD of the control distribution, may permit more appropriate comparisons of the responses of continuously distributed parameters. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report whenever the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making. CERHR calculated the values using the power or probit model and EPA Benchmark Dose Software version 1.3.2.
[b]Indications of no change (↔), significant increase (↑), or significant decrease (↓) based on ANOVA with post-hoc Dunnett test performed by CERHR.
[c]Malformation rates calculated by CERHR. No malformations were observed in controls. See Table 34 for malformations.
GD, gestational day.

Table 34
Malformation Incidence Rates in Offspring of Rats Injected Intraperitoneally with Hydroxyurea on Single GD or Orally Dosed on GD 6–15

Exposure, Gestational Day	7	8	10	11	12	13	14	6–15	6–15
Hydroxyurea dose (mg/kg bw)	750	750	750	750	750	750	750	300	450
External malformations									
Cranial defects	[a]	—	—	—	—	—	—	—	3/30
Facial defects	2/54	2/18	—	4/23	2/72	—	—	1/51	4/30
Craniofacial dysgenesis	—	—	—	—	—	—	—	3/51	2/30
Otocephaly	—	—	—	—	—	—	—	1/51	2/30
Ablepharia	—	1/18	—	2/23	1/72	—	—	—	—
Absent pinnae	—	—	—	—	—	—	—	—	3/30
Protruding tongue	—	—	—	—	—	—	—	—	1/30
Severe edema	—	—	1/9	3/23	1/72	—	—	—	—
Spina bifida	—	—	—	—	—	—	—	1/51	—
Abdominal wall defects	—	1/54	1/9	—	1/72	—	—	—	4/30
Amelia/phocomelia	—	—	1/9	—	—	—	—	—	—
Limb malrotations	—	—	—	—	1/72	—	—	—	5/30
Forepaw defects	—	—	—	—	2/72	24/80	—	—	—
Hindpaw defects	—	—	4/9	8/23	5/72	24/80	5/72	—	—
Tail defects	—	—	3/9	3/23	—	—	—	1/51	1/30
Visceral malformations									
Hydrocephalus	[a]	—	1/5	1/12	—	—	—	5/30	7/16
Eye defects	2/27	1/11	4/5	—	1/37	1/38	—	4/30	13/16
Cleft palate	1/27	1/11	—	2/12	7/37	2/38	—	—	—
Diaphragmatic hernia	—	—	—	—	3/37	—	—	—	—
Cardiovascular defects	1/27		5/5	2/12	1/37	—	—	1/30	2/16
Urogenital defects	—	1/11	—	1/12	—	—	—	—	2/16
Skeletal malformations									
Severe reduction of cranial bones	[a]	—	—	1/11	13/35	—	—	—	—
Reduced, misshapen facial bones	—	—	—	—	—	—	—	—	2/14
Dysgenesis of craniofacial bones	—	—	—	—	—	—	—	3/21	—
Reduction of orbital bones	—	—	—	—	—	—	—	—	5/14
Ectopic periotic bones	—	—	—	—	—	—	—	1/21	2/14
Reduced/absent/misshapen mandibula	—	—	—	—	—	—	—	2/21	6/14
Dysgenesis of									
Vertebrae/sternebrae/ribs	—	—	4/4	7/11	—	—	—	1/21	10/14
Long bones	—	—	4/4	2/11	4/35	—	—	—	—
Metacarpals/phalanges	—	—	—	—	2/35	34/42	—	—	—

From Aliverti et al. (1980).
Incidences of defects were presented as number fetuses affected/number examined. Malformations were not observed in the control group or in the 50 or 150 mg/kg bw/day hydroxyurea groups; rates of all malformations in GD 6 exposure group were ≤2%; no fetuses could be observed on GD 9 due to complete litter resorptions.
[a]When no information was entered by the study authors, it was assumed that no malformations were observed.
GD, gestational day.

routes during prenatal development. A time-response and dose-response study were conducted in Sprague-Dawley rats. In both studies, day of vaginal sperm was defined as GD 0. Dams were killed on GD 21, and implantation sites were examined. Fetuses were weighed and assessed for external malformations. Half the fetuses were examined for visceral malformation, and the other half were examined for skeletal malformations. **[No statistical analyses were conducted.]**

In the dose-response study, 30 dams/group were dosed orally with the 2% gum arabic vehicle and 10 dams/group were dosed orally with hydroxyurea at 50, 150, 300, or 450 mg/kg bw/day on GD 6–15. **[The specific method of oral dosing was not specified; gavage is assumed.]** Litters from 27 dams were examined in the control group, and 8–10 litters/group were examined in each treatment group. Results for non-malformation data in the dose-response study are summarized in Table 33. Postimplantation loss was increased at ≥300 mg/kg bw/day. Complete resorptions occurred in two of nine litters in the 300 mg/kg bw/day group and two of eight litters in the 450 mg/kg bw/day group. Fetal body weight was reduced at doses ≥150 mg/kg bw/day. Malformations were increased at ≥300 mg/kg bw/day; incidences of malformations observed in the 300 and 450 mg/kg bw/day group are summarized in Table 33. The types of malformations observed most commonly included hydrocephalus and defects in limb rotation, cranium and face, abdominal wall, eyes, vertebrae, and ribs. Gross abnormalities were not observed in limbs, but ossification was delayed.

In the time-response study, 10 rats/group were i.p. dosed with distilled water vehicle on GD 6–14 or 750 mg/kg bw hydroxyurea on GD 6, 7, 8, 9, 10, 11, 12, 13, or 14. In each dose group, litters from 7–10 dams were examined. High resorption incidence was observed when hydroxyurea was given on GD 7, 8, 9, 10, or 11 (postimplantation loss rates of 46.0–100% vs. 16.4% in

Table 35
Effects in Fetuses of Rats Exposed to Hydroxyurea by Intraperitoneal Injection

	Minimum dose (mg/kg bw)		At minimum teratogenic dose	
Treatment (GD)	Complete lethality	Teratogenicity	Fetal mortality (%)	Abnormal fetuses (%)
9	500	250	54	71
10	500	350	3	21
11	1000	500	3.5	98
12	2000	1000	64	100

From Murphy and Chaube (1964).
GD, gestational day.

control group). Exposure on GD 9 resulted in a 100% malformation rate. A marked reduction in fetal body weight was observed with hydroxyurea exposure on GD 8, 10, 11, or 12 **[decreased 27–37% compared to controls]**. Malformation rates were $\leq$1% in controls. Incidences of each malformation type in the hydroxyurea groups are summarized in Table 34. Abnormalities that were increased (with hydroxyurea exposure on a particular gestation day) involved the cardiovascular system (GD 10), eyes (GD 10 or 11) **[no increases were apparent after treatment on GD 11]**, palate (GD 12), diaphragm (GD 12), and limbs (GD 10, 11, 12, 13, or 14; especially GD 13). **[Skeletal defects were also observed in cranium (GD 12) and vertebrae and ribs (GD 10, 11).]** The study authors concluded that the rat genotype used in this study was highly susceptible to single i.p. doses of hydroxyurea.

The study authors noted differences in the spectrum of abnormalities with repeated versus single-day dosing with hydroxyurea. Craniofacial dysgenesis, hydrocephalus, microphthalmia/anophthalmia, and agnathia/micrognathia were frequently observed with repeated oral but not single i.p. dosing. **[It is not clear how authors reached conclusions about eye malformations from the data tables in the study.]**

Possible reasons stated by authors for increased incidence with repeated oral versus single i.p. dosing were that sublethal dosing allowed a larger number of fetuses to reach GD 21 or the occurrence of a cumulative effect. Malformed limbs and paws, cleft palate, and diaphragmatic hernia were rarely observed after repeated oral dosing but were observed with single-day i.p. dosing. Study authors noted the possibility that the threshold of effect was not reached in the repeat-dose study. The study authors concluded that the threshold level for embryotoxicity in this study was between 150 and 300 mg/kg bw/day.

Strengths/Weaknesses: This study used a good range of doses, permitting determination of low adverse effect level (LOAEL)/no adverse effect level (NOAEL) as well as benchmark dose. The authors showed the relative susceptibilities of various developmental stages (during the embryonic period) and produced a dose response for repeated oral dosing, including endpoints such as fetal weights. However, the malformation incidence is suspect due to the high number of resorptions (both with the single exposures and the higher, repeated exposures) noted in this study. In addition, it was not shown whether the vehicle used for the oral dosing portion may have affected absorption across the gastrointestinal tract. There was no visceral examination of control fetuses with repeated dosing of the vehicle (Table 3), although Tables 1 and 2 of the study suggest otherwise. The number of animals in the treated groups was low, and there was a lack of statistical analysis of the endpoints in this study. The oral dosing period was short, and there was no dosing during the fetal period, when cell division can still be occurring and the drug may have an effect. The lack of information on maternal toxicity is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this study is high for a dose-response for possible effects during the embryonic period. A NOAEL of 150 mg/kg/day by the oral route was noted.

Price et al. (1985b), under contract to the Chemical Industry Institute of Toxicology **[now CIIT Centers for Health Research]**, used hydroxyurea as a positive control in a study examining the developmental toxicity of dinitrotoluene in rats. Protocol and results for dinitrotoluene will not be discussed. Twenty-two F344 rats/group were gavaged with corn oil or 200 mg/kg bw/day hydroxyurea **[purity not reported]** on GD 7–20 (GD 0 = day of vaginal sperm). It appears that dams in this study were selected from three different breedings that were conducted to examine different dinitrotoluene doses. Based on previous studies, it was anticipated that the hydroxyurea dose would result in limited prenatal mortality and thus allow other types of toxicity to be observed. Dams were weighed and examined for clinical signs of toxicity during the study. Dams were killed on GD 20 and examined for implantation sites and body and organ weights. Fetuses were weighed, measured, sexed, and examined for external, visceral, and skeletal malformations. Hematologic analyses were conducted for dams and for one fetus/sex/litter. Statistical analyses included *t*-test for hematologic values and Mann-Whitney *U*-test and Fisher exact test for teratology data.

Twenty control and 19 hydroxyurea-treated dams had litters. The only effects reported for dams exposed to hydroxyurea were reduced red blood cell count and hematocrit levels. **[Data and levels of statistical significance were not shown by the study authors.]** Effects observed in fetuses were decreased body weight, crown-rump length, reticulocyte count, red blood cell count, and hematocrit and increased red blood cell size and red blood cell distribution width. **[The study authors did not include data for the fetal effects described above but stated that the data were available in an unpublished report.]** Significant increases were observed for numbers of litters containing fetuses with external malformations (9/19 vs. 0/20 in controls), visceral malformations (5/19 vs. 0/20), skeletal malformations (10/19 vs. 3/20), and total malformations (13/19 vs. 3/20). Types of

malformations observed most frequently were meningocele, exencephaly, anophthalmia, and fused cervical arches. Mean total malformed fetuses/litter was 30.6% in the hydroxyurea group and 3.8% in the control group. The study authors concluded that 200 mg/kg bw/day hydroxyurea administered orally was an excellent positive control for maternal and offspring toxicity in F344 rats.

Strengths/Weaknesses: The methods and results were well described. The dose level and dosing period selection were appropriate and a large number of animals/group allowed for meaningful analysis of the results. The observed maternal toxicity was reported in detail. However, because this study used hydroxyurea as a positive control, only one dose level was used. The vehicle control used for comparison received corn oil, although the vehicle used for the hydroxyurea dosing solution was water. The malformation incidence for specific terata was not provided, only the overall malformation incidences were provided.

Utility (Adequacy) for CERHR Evaluation Process: This study has utility in that it provides a known effect level for hydroxyurea after exposures during the embryonic and fetal periods. It does not, however, provide useful information for identifying a NOAEL or LOAEL.

Price et al. (1985a), under contract to the Chemical Industry Institute of Toxicology **[now CIIT Centers for Health Research]**, used hydroxyurea as a positive control in a study to examine teratogenicity and postnatal effects of aniline hydrochloride in rats. The protocol and results for aniline hydrochloride will not be discussed. On GD 7–20 (GD 0 = day of vaginal sperm) 24 F344 rat dams were gavaged with distilled water vehicle and 27 dams were gavaged with 200 mg/kg bw/day hydroxyurea **[purity not reported]**. The hydroxyurea dose was based on results of a dose-range finding study. Dams were weighed and examined for clinical signs of toxicity during the study and were then killed on postnatal day (PND) 20. Implantation sites were examined, and hematologic analysis was conducted in dams and one fetus/sex/litter. Fetuses were, sexed, weighed, measured, and examined for external, visceral, and skeletal malformations. Dams or litters were considered the experimental unit in statistical analyses. Statistical analyses included Mann-Whitney *U*-test and Fisher exact test for fetal malformations, and *t*-test for hematologic values.

On GD 20, there were 22 dams in the control group and 25 in the hydroxyurea group. **[With the exception of external, visceral, and skeletal malformation rates, no data were shown by study authors.]** Significant effects observed in the hydroxyurea group were decreased maternal weight gain, increased percentages of resorbed implants, and decreased live fetuses. Hematologic changes observed in dams of the hydroxyurea group included decreased red blood cell count, decreased hematocrit, increased mean corpuscular volume, and increased red cell distribution width. Effects in fetuses exposed to hydroxyurea included decreased body weight, crown-rump length, relative spleen weight, and placental weight. Hematologic alterations reported in fetuses from the hydroxyurea group included decreased red blood cell count, increased mean corpuscular volume, and increased red cell distribution width. Exposure to hydroxyurea increased incidences of litters containing fetuses with external malformations (14/25 vs. 2/22 in controls), visceral malformations (6/25 vs. 0/22), skeletal malformations (14/25 vs. 1/22), and total malformations (16/25 vs. 3/22). Malformations consisted mainly of hydrocephalus, anophthalmia, meningocele, and fused cervical arches.

Price et al. (1985a) also used hydroxyurea as a positive control in a study to examine postnatal effects after prenatal exposure of rats to aniline hydrochloride. The dosing schedule was from GD 7 through parturition. **[No information was provided on the number of dams treated.]** The day pups were born was designated PND 0. On PND 0, pups were counted, sexed, weighed, measured, and examined for viability, gross defects, and clinical signs of toxicity. Litters were culled to eight pups, with equal numbers of each gender when possible. Pups were weighed at various time points from PND 0–60. Dams were killed on PND 30 for assessment of hematology endpoints and organ weights. One pup/litter was killed on PND 0, 10, 25, or 50 to measure liver and spleen weights and conduct hematologic evaluations. Remaining pups were killed on PND 60 for measurement of liver, spleen, and testis weight. Maturational landmarks (e.g., neurobehavioral landmarks, pinna detachment, puberty) were evaluated in pups during the postnatal period. Statistical analyses appeared to be similar to those conducted in the prenatal toxicity test.

Results in the hydroxyurea group included decreased numbers of live pups at birth, increased incidence of malformations (e.g., hydrocephalus, exencephaly, anophthalmia, microphthalmia, and meningocele), decreased birth weight of male pups, decreased body weights in offspring of both sexes during the postnatal period, decreased relative weights of offspring liver and spleen (on PND 0, 10, 25, 50, and 60), and decreased relative testis weight on PND 60. Most offspring hematologic values were within control ranges during the postnatal period, with the exceptions of increased mean corpuscular volume on PND 0 and elevated methemoglobin on PND 50. Developmental delays were observed for vaginal opening, testis descent, and wire-grasping ability. Effects observed in dams killed on PND 30 included increased mean corpuscular volume, reduced red blood cell count, and decreased relative liver weight. **[No data were shown by study authors for the postnatal study of hydroxyurea.]** The study authors concluded that oral exposure to 200 mg/kg bw/day hydroxyurea is an excellent positive control for maternal toxicity and pre- and postnatal offspring toxicity in F344 rats.

Strengths/Weaknesses: The methods and results were well described. The dose level and dosing period selection were appropriate, and a large number of animals per group allowed for meaningful analysis of the results. The maternal toxicity observed was reported in detail. However, because this study used hydroxyurea as a positive control, only one dose level was used. The vehicle control used for comparison received corn oil, although the vehicle used for the hydroxyurea dosing solution was water. The malformation incidence for specific terata was not provided; only the overall malformation incidences.

Utility (Adequacy) for CERHR Evaluation Process: This study has utility in that it provides a known

Table 36
Developmental Toxicity in Rats Intraperitoneally Dosed with Hydroxyurea at Various Doses and Gestational Days

Dosing regimen (mg/kg bw)	Fetal mortality (%)	Abnormal/total fetuses
Single undivided doses		
GD 9		
185	No effect[a]	16/30
250[b]	54, unaffected	25/35 **[71%]**, 53%
375	80	6/6
GD 10		
250	No effect	13/72
375[b]	33, 63	3/8 **[38%]**, 37%
400	34	8/25
GD 11		
375	No effect	23/91
500[b]	No effect	29/30 **[97%]**, 98%
GD 12		
750[b]	No effect	29/30 **[97%]**, 96%
1000[b]	63, 64	20/20, 100%
Divided doses[c]		
GD 9		
375 in two divided doses	64	21/21
500 in two divided doses	96.5	2/2
GD 10		
375 in two divided doses	No effect	6/43
500 in two divided doses	97.5	1/1
GD 11		
500 in two divided doses	No effect	23/37
750 in two divided doses	19	17/17
GD 12		
1000 in two divided doses	100	
1500 in two divided doses	100	
Administered on multiple days		
125 (GD 9)+165 (GD 10)	17	0/39
185 (GD 9)+250 (GD 10)	No effect	13/37
165 (GD 10)+250 (GD 11)	14	0/46
250 (GD 10)+375 (GD 11)	21	2/36
125 (GD 9)+165 (GD 10)+250 (GD 11)	11	11/37
185 (GD 9)+250 (GD 10)+375 (GD 11)	96	1/1
250 (GD 11)+500 (GD 12)	33	19/22
375 (GD 11)+750 (GD 12)	90	4/4
165 (GD 10)+250 (GD 11)+500 (GD 12)	34	27/33
250 (GD 10)+375 (GD 11)+750 (GD 12)	93	7/7
125 (GD 9)+165 (GD 10)+250 (GD 11)+500 (GD 12)	35	25/29
185 (GD 9)+250 (GD 10)+375 (GD 11)+750 (GD 12)	100	

From Chaube and Murphy (1966).
[a]↔, Within control values of 0–10%.
[b]The same doses were examined in two separate experiments, the results of which are separated by commas.
[c]Dose indicated is the total dose.
GD, gestational day.

effect level for hydroxyurea after exposures during the embryonic and fetal periods. However, it does not provide useful information in identifying a NOAEL or LOAEL.

3.2.1.2 Parenteral dosing—general prenatal developmental toxicity endpoints: This section includes studies focusing on prenatal toxicity endpoints in rats parenterally exposed to hydroxyurea. Studies providing possible dose-response information are presented before studies examining the effects of single dose levels. In each case, studies are presented in the order in which they were published.

Murphy and Chaube (1964), support not indicated, examined the effects of hydroxyurea exposure on developmental toxicity in rats. Three other compounds were examined but will not be discussed here. Wistar rats were i.p. injected with hydroxyurea at 0 (distilled water vehicle) or 50–2000 mg/kg bw on GD 9, 10, 11, or 12. **[No information was provided on hydroxyurea purity, the number of dams exposed, specific doses administered, or day of vaginal plug.]** Animals were inspected and weighed during the study and killed on GD 21. Fetuses (20–54/group) were examined for gross and skeletal anomalies. **[It does not appear that statistical analyses were conducted.]** No signs of toxicity were observed in dams. Effects in fetuses are summarized in Table 35. The study authors reported that no fetal effects were observed at doses <250 mg/kg bw/day. The minimum teratogenic dose and the dose resulting in 100% mortality were lower earlier in gestation. Malformations varied by day of exposure. Types of malformations present in the majority of fetuses

(treatment day that resulted in the majority of fetuses affected) included exencephaly (GD 9 and 12), cleft palate (GD 11 and 12), harelip (GD 9), micrognathia (GD 9), retarded clubbed foreleg (GD 12), retarded clubbed rear leg (GD 9, 11, 12), ectrodactyly of fore- or hind-paw (GD 11 and 12), or retarded tail (GD 11 and 12). **[Actual numbers of affected fetuses were not reported.]** The study authors claimed that prior administration of thymidine, guanine, adenine, or citrovorum failed to protect the fetuses against malformations. **[No information was provided about the protocol for that part of the experiment, and data were not shown.]** The study authors concluded that hydroxyurea induced gross and skeletal abnormalities in the rat fetus.

Strengths/Weaknesses: Strengths are the conduct of the study on different days of gestations and the description of types of malformations. Weaknesses are the small sample size; the poor reporting of results, without the specific incidence of the individual malformations and without statistical analysis; poor dose selection; and inadequate evaluation or reporting of maternal toxicity.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Chaube and Murphy (1966), supported by the American Cancer Society, the Albert and Mary Lasker Foundation, and NIH, examined developmental toxicity in rats exposed to hydroxyurea during prenatal development. Additional compounds were tested but will not be discussed here. Wistar rats were i.p. injected with hydroxyurea **[purity not reported]** at the doses and gestation days listed in Table 36. Control rats were injected with saline. The day after mating was considered GD 0. Rats were killed on GD 21. Implantation sites were examined, and fetuses were assessed for viability and gross malformations. Skeletal malformations were examined in two-thirds of the litter if there were gross malformations or in the entire litter if there was no evidence of gross malformations.

In a study examining maternal mortality, no deaths occurred in dams exposed to $\leq$2000 mg/kg bw hydroxyurea on GD 11. The LD_{50} for dams on GD 11 was estimated at $>$4700 mg/kg bw. Dosing regimen and results for studies in which hydroxyurea was administered once between GD 9 and 12 are summarized in Table 36. **[From the text in the report, it appears that more doses may have been tested, but results were provided only for doses that induced malformations.]** The lowest doses to result in 100% fetal resorption were estimated at 500 mg/kg bw on GD 9 and 10, 750 mg/kg bw on GD 11, and 1500 mg/kg bw on GD 12. Depending on the day of exposure, malformations were observed at doses $\geq$185 mg/kg bw. Table 36 also lists results of studies in which hydroxyurea was administered on a single gestation day in divided doses given 4 hr apart. The study authors concluded that administering hydroxyurea in divided doses decreased lethality and abnormalities on GD 10 and 11 and increased lethality on GD 12. Results of testing in which hydroxyurea was administered on multiple days are also included in Table 36. The study authors concluded that the effects appeared to be cumulative and varied according to the total dose. Malformations were reported to be more severe with multiple versus single dosing.

In the study where a single, undivided dose was administered, malformations (day of exposure resulting in malformations) included exencephaly and cleft lip (GD 9), encephalocele (GD 12), cleft palate (GD 9, 11, and 12), micrognathia (GD 9 and 12), and retardation of body and tail and deformed appendages (GD 9, 10, 11, and 12). In addition to the gross abnormalities, examination of the skeleton showed defects in vertebrae, ribs, sternebrae, and cranium. In the study where the total dose was administered 4 hr apart on a single day of gestation, malformations observed depended on dose. At the highest doses, malformations (day of exposure resulting in malformations) included exencephaly (GD 9), encephalocele (GD 11), cleft palate/lip and micrognathia (GD 9 and 11), and deformed appendages and tail defects (GD 9, 10, 11). Malformations could not be evaluated for GD 12 because of complete resorptions.

The study authors concluded that in the rat, development toxicity after prenatal hydroxyurea exposure occurred at dose levels one-tenth to one-third those causing lethality in the dam.

Strengths/Weaknesses: The study examined the lethality endpoint quite thoroughly, with effects observed at different stages of development, allowing for a good dose-response curve for lethality for specific days of gestation to be developed. The types of malformations were well described; however, pups were assigned to skeletal evaluation (possibly missing visceral endpoints), and the dosing period did not encompass the full period of embryogenesis or the fetal period. The fetus, as opposed to the litter, was the unit of analysis, and the number of litters available for examination was quite small.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Scott et al. (1971), supported by NIH and the Pharmaceutical Manufacturers Association Foundation, examined the effects of hydroxyurea exposure on teratogenesis and inhibition of DNA synthesis in rat embryos. Two sets of experiments were conducted in Wistar rats. In both experiments, the rats were i.p. injected on GD 12 (GD 0 = day of vaginal sperm) with hydroxyurea **[purity not reported]** in aqueous solution at 250, 500, 750, or 1000 mg/kg bw/day. Results in hydroxyurea-treated rats were compared to historic control data obtained over the last 4 years in the authors' laboratory. **[No information was provided on number of dams treated/group, and no statistical analyses were reported.]** In the first experiment, pregnancies were terminated on GD 20. Implantation sites were counted, and all live fetuses were weighed and examined for external malformations. Some fetuses were examined for skeletal malformations, and others were examined for visceral malformations. In a second experiment, rats were i.p. injected with thymidine/^{3}H-thymidine at 3, 6, 9, 15, 21, or 27 hr after hydroxyurea treatment. Two hr after thymidine injection, one uterine horn was removed from at least three pregnant rats/group. All but one embryo/litter were sonicated, and radioactivity levels were measured by liquid scintillation counting. One embryo/litter was examined histologically to determine the extent of cell death. Dams were allowed to continue their pregnancies until GD 20, at which time they were killed and fetuses and implantation sites were examined. Using a spectrophotometry method, hydroxyurea levels were measured in maternal plasma and embryos in at least three dams and four embryos/group. **[It was not**

Table 37
Effects in Rat Fetuses After Exposure of Dams to Hydroxyurea by Intraperitoneal Injection on GD 12

Dose, mg/kg bw	Dead/ resorbed (%)	Survivors malformed (%)	Fetal weight (% of controls)
Experiment 1 (no thymidine exposure)			
0	4	2	
250	4	1	97
500	10	8	92
750	11	69	75
1000	28	100	56
Experiment 2 (thymidine exposure)			
0	9	0	
250	11	3	97
500	9	14	80
750	8	86	59
1000	25	100	54

From Scott et al. (1971).
GD, gestational day.

stated if the hydroxyurea measurements were made in one of the experiments described above.]

Effects of hydroxyurea on fetal survival, malformations, and weights observed in both sets of experiments are summarized in Table 37. In both experiments, hydroxyurea induced dose-related increases in percentages of fetal malformations and dose-related decreases in fetal weights. Percentages of dead or resorbed fetuses were consistently increased only at the 1000 mg/kg bw/day dose. The types of fetal abnormalities reported were ectrodactyly, hydrocephalus, micrognathia, short kinky tail, cleft palate, hydroureter, hydronephrosis, fused or wavy ribs, anal atresia, diaphragmatic hernia, hypoplastic lungs, cardiac and aortic arch defects, exophthalmia, and cranial dysplasia. Exposure to hydroxyurea doses $\geq$500 mg/kg bw/day resulted in severe inhibition of DNA synthesis, as indicated by radiolabel intake, from 5–23 hr after hydroxyurea injection. Exposure to 250 mg/kg bw/day hydroxyurea resulted in reduced DNA synthesis at $\leq$5 hr, followed by a rebound in DNA synthesis at 8 hr, and a return to control levels at 11 hr after hydroxyurea injection. The study authors noted that as the hydroxyurea concentration in the embryo was reduced to 5×10^{-4} M **[38 mg/L]**, the rate of DNA synthesis began to increase. The authors stated that 10^{-4} to 10^{-3} M **[7.6–76.1 mg/L]** represented the critical concentration for inhibitory effects. At 5 hr after injection of 750 mg/kg bw hydroxyurea, extensive cell death was observed in mesoderm of limb bud and neural tube.

The study authors concluded that at least three effects of hydroxyurea could be identified from this study: a rapid and profound reduction in DNA synthesis; a synchronization of embryonic cells characterized by initial decreases in DNA synthesis followed by rebound effects; and induction of embryonic cell death. Some of the data from this study were presented in additional publications by these authors (Ritter et al., 1973; Wilson et al., 1977). Data from an additional study were presented in one of the publications (Ritter et al., 1973), and those data are described below.

[Analysis by CERHR of the per-implant and per-fetus data underlying Table 37 using Fisher exact test showed statistical significance to be attained for dead/resorbed implants and for malformed survivors at $\geq$500 mg/kg bw hydroxyurea without thymidine. Using a probit model, for dead/resorbed implants the BMD_{10} is 637 mg/kg bw and the $BMDL_{10}$ is 565 mg/kg bw. For malformed survivors, the BMD_{10} is 365 mg/kg bw and the $BMDL_{10}$ is 333 mg/kg bw. The Expert Panel notes that per-litter analysis would have been preferable for these data.]

Strengths/Weaknesses: The study included multiple doses and produced data supporting a good dose-response relationship for the endpoints examined. The study also describes a possible mechanism of action for hydroxyurea, and the mechanistic investigation is a strength of the study. However, a single day of gestation (GD 12) was evaluated to examine the outcome and specific data on the terata observed were not provided, only an overall effect level. The lack of appropriate statistical analysis is an additional weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Ritter et al. (1973), published a report that reiterated some of the data originally presented in the study by Scott et al. (1971), described above. The hydroxyurea data were provided to compare effects observed with cytosine arabinoside, which will not be discussed here. In addition, new data were reported for hydroxyurea. The new data comprised numbers of digits missing from limbs of 20-day-old rat fetuses after exposure to 1000 mg/kg bw hydroxyurea on GD 12 compared to GD 13. **[It is not clear whether the GD 12 data were obtained from rats exposed in the study by Scott et al. (1971); no experimental details were given for studies involving exposure on GD 12 or GD 13.]** There were more digits missing from forelimbs after exposure on GD 13 than on GD 12. With GD 12 exposure, 91% of fetal forelimbs were missing one digit and none of the limbs were missing more than one digit. After GD 13 exposure to hydroxyurea, 4–6% of forelimbs had one or two missing digits, 11% had three missing digits, 30% had four missing digits, and 49% had five missing digits.

Strengths/Weaknesses: This study produced useful information on limb malformations induced by hydroxyurea, showing the critical period for missing forelimb digits. However, the study used a single dose level on 2 separate days of gestation, provided no useful dose-response information, and evaluated only one malformation type.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited-to-no utility for the evaluation.

Additional effects on prenatal developmental toxicity were reported by Theisen (1979), which is summarized in Section 3.2.1.5.

Asano and Okaniwa (1987), support not indicated, examined developmental toxicity of hydroxyurea in two rat strains. Effects on prenatal development are described here, while effects on postnatal development are described in Section 3.2.1.5. On GD 9–12 (GD 0 = day of vaginal sperm), 15–16 Sprague-Dawley rats/group were i.p. injected with 0, 100, or 200 mg/kg bw hydroxyurea **[purity not reported]** and five Wistar rats/group were i.p. injected with 0, 25, 50, 100, or 200 mg/kg bw hydroxyurea. Controls were injected with saline vehicle. Dams were killed on GD 21 and examined for implantation sites. Fetuses were assessed for viability and were sexed, and weighed. All Sprague-Dawley fetuses and all

Wistar fetuses from the 200 mg/kg bw group were examined for malformations. One-third of Wistar fetuses in the control and three lower dose groups were examined for malformations. Statistical analyses included Kruskal-Wallis nonparametric one-way ANOVA, one-way ANOVA, or Wilcoxon rank test.

Exposure to hydroxyurea had no effect on numbers of implantation sites, resorptions, or live fetuses in either strain. Fetal body weights were significantly reduced in both rat strains in the 200 mg/kg bw/day group **[by ~7% in Sprague-Dawley rats and ~20% in Wistar rats, compared to respective controls. CERHR estimates of benchmark doses (mg/kg bw/day) for body weights in Sprague-Dawley rats were BMD_{10} 203, $BMDL_{10}$ 187, BMD_{1SD} 201, and $BMDL_{1SD}$ 168. CERHR estimates of benchmark doses (mg/kg bw/day) for body weights in Wistar rats exposed to the two highest doses (the only data shown) were BMD_{10} 120, $BMDL_{10}$ 81, BMD_{1SD} 79, and $BMDL_{1SD}$ 42.]** Malformation rates in the control and 100 mg/kg bw/day groups were 1.1% in Sprague-Dawley rats and 6.7–10% in Wistar rats. In male and female fetuses of the high-dose group, malformation rates were significantly increased to 43.8–51.1% in Sprague-Dawley rats and 86.8–88.6% in Wistar rats. The malformations observed most commonly in both strains of rats were dilation of lateral ventricle, anophthalmia, microphthalmia, and ventricular septal defect. Exencephaly, cleft palate, and micrognathia were also observed in high-dose Wistar males. **[Using per-fetus data for malformation incidence in Sprague-Dawley rats, the BMD_{10} (mg/kg bw/day) was 171 for males and 134 for females, and the $BMDL_{10}$ was 133 for males and 126 for females. For malformation incidence in Wistar rats, again based only on the two high-dose levels and the control, the BMD_{10} (mg/kg bw/day) was 156 for males and 153 for females, and the $BMDL_{10}$ was 97 for males and 990 for females. The Expert Panel notes that a litter-based analysis would have been preferred.]** The study authors concluded that prenatal exposure of rats to hydroxyurea 200 mg/kg bw/day resulted in growth retardation, pup mortality, and malformations. In a comparison of the two rat strains, the study authors noted higher rates of malformations and stillbirths in Wistar rats than in Sprague-Dawley rats.

Strengths/Weaknesses: This study compared the effect of hydroxyurea exposure on different strains of rats using a reasonable range of dose levels; however, group sizes were small. The incidence of malformation within each strain was very well described within the study. It is unclear if additional findings would have occurred if the authors had expanded the short dosing period, which did not cover the entire period of embryogenesis and the fetal period. The use of unequal group sizes made it difficult to determine if the incidence of effects observed was due to the power of detecting an effect at that specific dose level on that specific day of gestation.

Utility (Adequacy) for CERHR Evaluation Process: This study contributes important information for effects at the lower end of the dose-response curve for these specific days of gestation and as such, has utility in this evaluation.

Soukup et al. (1967), supported by NIH, examined hydroxyurea developmental toxicity in rats. The focus of the study was genetic toxicity, which is described in Section 2.4.2. A ''commercial strain'' of rats was injected with hydroxyurea **[purity not given]** at 750 mg/kg bw on GD 13 (day of vaginal sperm = GD 1), and fetuses were examined on GD 21. A control group was exposed to the saline vehicle. **[No other information was provided, such as numbers of treated dams or specific method of injection.]** All hydroxyurea-exposed fetuses were small,

Table 38
Developmental Toxicity in F344 and Wistar Rats Exposed to 500 mg/kg bw Hydroxyurea by Intraperitoneal Injection on GD 11

Endpoint	F344 rat	Wistar rat
Maternal weight gain	↓**[31%]**	↔
Male fetus weight[a]	↓26% **[32%]**	↓31%**[25%]**
Female fetus weight[a]	↓26% **[30%]**	↓31%**[28%]**
Male fetus length[a]	↓15% **[19%]**	↓18% **[16%]**
Female fetus length[a]	↓15% **[18%]**	↓18% **[15%]**
Fetal resorptions	↑4-fold	↔
Litters with resorptions or deaths	↑2-fold	↔[b]
Cleft palate	↔	↑ (8 fetuses/5 litters vs. 0)
Enlarged brain ventricles	↔	↑ (12 fetuses/6 litters vs. 3 fetuses/1 litter)
Sternebral variations	↑ (45 fetuses/14 litters vs. 1 fetus/1 litter)	↔
Skull anomalies	↑ (53 fetuses/14 litters vs. 0)	↑ (42 fetuses/8 litters vs. 0)
Limb anomalies	↑ (12 fetuses/7 litters vs. 0)	↑ (25 fetuses/6 litters vs. 0)
Rib anomalies	↑ (38 fetuses/13 litters vs. 0)	↑ (13 fetuses/7 litters vs. 1 fetus/1 litter)
Vertebral anomalies	↑ (45 fetuses/14 litters vs. 0)	↑ (45 fetuses/9 litters vs. 0)
Vertebral centrum variations	↑ (53 fetuses/14 litters vs. 42 fetuses/11 litters)	↑ (33 fetuses/8 litters vs. 2 fetuses/2 litters)
Vertebral centrum anomalies	↑ (11 fetuses/6 litters vs. 0)	↑ (15 fetuses/6 litters vs. 0)

From DePass and Weaver (1982).
[a]Changes in fetal weight and length according to author calculations presented in the results section **[CERHR calculations follow bold brackets]**. It seems that the study authors may have reversed some of the fetal weight and length effects per strain in either Table 2 of the study or in the text of the results section.
[b]Resorptions or deaths occurred in 60% of treated litters compared to 30% of controls; there was no statistically significant difference.
↑,↓, Statistically significant increase or decrease, respectively, compared to control for each strain; ↔, no statistically significant effect.
GD, gestational day.

and 92% had gross malformations. The types of abnormalities observed included cleft palate, tail and head defects, syndactyly, malpositioned limbs, and short mandible.

Strengths/Weaknesses: Weaknesses are the use of a single dose level, administration on a single day of gestation, and lack of detail on the number of dams and strain of rat.

Utility (Adequacy) for CERHR Evaluation Process: This study has no utility in the evaluation. It used a limited dosing paradigm, and it was unlikely to detect anything other than effects due to massive cell death.

DePass and Weaver (1982), support not indicated, compared hydroxyurea-induced teratogenicity in two strains of rats. The effects of aspirin were also examined but will not be discussed here. On GD 11 (GD 0 = day of vaginal plug or vaginal sperm), 10–15 Wistar or F344 rats/group/strain, were i.p. injected with saline vehicle or 500 mg/kg bw hydroxyurea **[purity not reported]**. Rats were killed on GD 20. Fetuses were examined for viability, and were weighed, measured, and assessed for gross abnormalities. Half of the fetuses were examined for visceral defects, and the other half were assessed for skeletal abnormalities. The litter was the experimental unit in statistical analyses. Continuous data were analyzed by *t*-test, discontinuous data were analyzed by rank-sum procedure, and frequency data were analyzed by Fisher exact test.

Study results are summarized in Table 38. Exposure to hydroxyurea resulted in decreased maternal weight gain in F344 rats and decreased fetal weight and length in both strains. Resorptions and fetal deaths were increased in F344 rats. Although more resorptions were reported in treated than in control Wistar rats, the effect did not achieve statistical significance. Fetal abnormalities were increased in both rat strains. Increases in cleft palate and enlarged brain ventricles were only observed in Wistar rats from the hydroxyurea group. Increases in sternal variations were observed only in F344 rats exposed to hydroxyurea. In both strains, hydroxyurea exposure was associated with increases in anomalies of skull, limbs, ribs, and vertebrae. Skull and limb abnormalities were characterized by incomplete ossification. Rib and vertebrae anomalies included extra, missing, or fused vertebrae and missing or malformed ribs. The study authors concluded that the two strains of rat were equally sensitive regarding most hydroxyurea-induced developmental toxicity endpoints. However, Wistar rats were more sensitive to soft tissue malformations, such as cleft palate.

Strengths/Weaknesses: Strengths are the comparison of effects of hydroxyurea exposure in two different stains of rats and the description of maternal toxicity. Weaknesses are the single, relatively high dose level used on a single day of gestation and the summary of malformation data without the provision of individual malformation rates.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited utility.

Maronpot et al. (1983), support not indicated, used hydroxyurea as a positive control in a study on the developmental toxicity of ethylene glycol. The ethylene glycol results will not be discussed here. On GD 11 (GD 0 = day of vaginal plug), 20 F344 rats were i.p. injected with 500 mg/kg bw hydroxyurea **[purity not reported]** in saline. Twenty dams in the negative control group were not treated. Dams were killed on GD 21. Half the fetuses in each litter were examined for visceral and head malformations, and all fetuses were examined for skeletal malformations. Statistical analyses included Bartlett *t*-test for homogeneity of variance, Duncan multiple range test, paired group F_{max} test, Cochran *t*-test, Student's *t*-test, Weil method, multiple sum of ranks test, χ^2 test, and Fisher exact test. **[It did not appear that statistically significant findings for hydroxyurea were discussed in the results section.]** Major malformations were observed in 100% of treated litters compared to 25% of control litters. Malformations were observed most commonly in limbs, tail, ribs, vertebrae, skull, and cardiovascular system. The study authors concluded that the results in rats treated with hydroxyurea confirmed the suitability of the F344 rat for teratogenicity testing.

Strengths/Weaknesses: This study provides a very good description of the different malformations that were observed after exposure to a single, high dose of hydroxyurea on a single day of gestation, with an adequate number of animals. However, it provides no information useful for a dose-response curve or for effects on other days of gestation.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited utility.

Spencer et al. (2000), supported by NIH, examined developmental toxicity in rats exposed to hydroxyurea. Decidualization responses in pregnant rats were also examined and are discussed in Section 4.2. Five Sprague-Dawley rats/group were i.p. injected with saline vehicle or 500 mg/kg bw hydroxyurea (98% purity) on GD 5–8 (day of vaginal sperm = GD 1). Rats were killed on GD 16 for assessment of corpora lutea, implantation sites, placental weight, and fetal viability and weight. Data were analyzed by Student's *t*-test and ANOVA. Exposure to hydroxyurea resulted in increased numbers of dead or resorbed fetuses (mean $\pm$ SEM 7.1 $\pm$ 0.7 in treated, 0.2 $\pm$ 0.1 in control) and increased post-implantation loss (mean $\pm$ SEM 94.9 $\pm$ 3.0% in treated, 2.8 $\pm$ 1.7% in control). Hydroxyurea induced decreases in placental weight (mean $\pm$ SEM 0.1 $\pm$ 0.1 g treated, 4.4 $\pm$ 0.3 g control), live fetal weight (mean $\pm$ SEM 0.1 $\pm$ 0.1 g treated, 4.1 $\pm$ 0.2 g control), and numbers of live fetuses/litter (mean $\pm$ SEM 0.2 $\pm$ 0.2 treated, 14.0 $\pm$ 0.8 control). The study authors concluded that developmental processes dependent on decidual homeostasis were affected by hydroxyurea exposure.

Strengths/Weaknesses: The idea of examining several cellular processes that may be affected by hydroxyurea is interesting, and the evaluation of placental weight is a strength. However, the study used a single high dose, with a limited exposure period. The small number of animals and the early assessment on GD 16 are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study does not contribute to determining a NOAEL and, therefore, has limited utility for the evaluation.

3.2.1.3 Parenteral studies examining possible mechanisms of prenatal developmental toxicity: This section focuses on studies that examine possible mechanisms of hydroxyurea-induced prenatal developmental toxicity in rats. The studies in this section are arranged according to the following topics:

- Time specificity of developmental toxicity;
- Effects of circadian rhythms on developmental toxicity;
- Effects related to cell proliferation or DNA or ribonucleic acid (RNA) synthesis;

Table 39
Developmental Toxicity in Rats Treated with Hydroxyurea on Different Gestation Days

	Day of hydroxyurea exposure						
Endpoint	GD 8	GD 9	GD 10	GD 11	GD 12	GD 13	GD 14
Postimplantation loss[a]	↑ to 84.9%	↑ to 100%	↑ to 90.2%	↑ to 76.5%	↔	↔	↔
Fetal weight	↓37%	No data	↓31%	↓30%	↓37%	↔	↔
Malformations[b]							
External[c]	11.11%	No data	55.5%	30.43%	15.27%	75%	8.3%
Visceral[d]	18.18%	No data	100%	33.3%	23%	7.89%	0
Skeletal[e]	0	No data	100%	72.7%	48.5%	80.9%	0
Total[f]	16.6%	No data	100%	52.17%	37.5%	75%	8.3%

From Giavini et al. (1979).
[a]Rate of postimplantation loss in controls was 16.4%.
[b]No statistical analyses appear to have been conducted for malformations.
[c]Incidence of external malformations was reported at 1.3% in controls.
[d]No visceral malformations were observed in controls.
[e]No skeletal malformations were observed in controls.
[f]Incidence of total malformations was reported at 1.3% in controls.
↑,↓, Statistically significant increase or decrease compared to controls, respectively; ↔, no statistically significant effects compared to controls. GD, gestational day.

- Effects related to cartilage or bone development;
- Effects of direct compared to indirect exposure; and
- Effects on androgen-induced masculinization.

Giavini et al. (1979) examined the effect of treatment day on hydroxyurea-induced developmental toxicity in rats. **[The study was published in Italian but included an English abstract. Details were obtained from the text of the Methods and Results section and from the tables translated by A. Iannucci.]** Sprague-Dawley rats (n = 7–10/group) were i.p. dosed with 750 mg/kg bw hydroxyurea on GD 8, 9, 10, 11, 12, 13, or 14 (day of vaginal sperm = GD 0). Controls were i.p. injected with the physiological solution used as vehicle on GD 8–14. Dams were killed on GD 21. Implantation sites and fetuses were examined. Live fetuses were weighed and examined for external, visceral, and skeletal malformations. **[There did not appear to be a discussion of statistical analyses.]**

Major study findings are summarized in Table 39. Increases in postimplantation loss occurred after exposure on GD 8–11, and decreased fetal weights were observed with exposure on GD 8, 10, 11, and 12. Although it was not clear if malformation data were statistically analyzed, the data in Table 39 suggest that exposure to hydroxyurea increased total, external, skeletal, and visceral malformations compared to the ≤1.3% rate in controls. Malformations of the fore- and hindlimbs occurred at the highest incidence. Skeletal malformations were consistent with external malformations. Cleft palate and malformations of the cardiovascular and nervous system, eye, and tail were also observed. According to study authors, rats were most susceptible to cardiac malformations after exposure on GD 10, to cleft palate after exposure on GD 12, and to fore- and hind-limb malformations after exposure on GD 13.

Strengths/Weaknesses: Strengths are the comparison of effects on different days and a good description of malformations; however, the hydroxyurea dose was high, and the control group was inappropriate because they were dosed multiple times over several days.

Utility (Adequacy) for CERHR Evaluation Process: The use of a single, extremely high dose level of hydroxyurea on 1 day of gestation limits the useful information proved by this study, although it can be used to support assessment of the critical period for malformations.

Additional information on time-specificity after i.p. dosing is available from Aliverti et al. (1980), which is summarized in Section 3.2.1.1.

Clayton et al. (1975), support not indicated, examined circadian effects on hydroxyurea-induced developmental toxicity in rats. Sprague-Dawley rats were assigned randomly to groups and mated at 4-hr intervals (0000, 0400, or 0800 of the light phase and 1200, 1600, or 2000 of the dark phase). On GD 12 (GD 0 = day of breeding), females were i.p. injected with saline vehicle (n = 18) or 750 mg/kg bw hydroxyurea **[purity not given]** (n = 42; 1–9/time period) during the same circadian phase at which they were mated. Developmental ages of fetuses were 288 ± 2 hr at the time of exposure. After injection, activity was monitored in 8 rats **[apparently only from the hydroxyurea group]**. Pregnant rats were killed just before parturition (GD 20 or 21), and fetuses were examined for external malformations. Litter means were considered single dependent measures. Data were analyzed by a cosinor method to estimate the phase of greatest effect. **[The cosinor method uses a least-square approximation of a time series using a cosine function of a known period, in this case, 24 hr.]** Regression analyses were conducted to identify possible relationships between malformations and activity during the first 8 hr after hydroxyurea exposure.

A single resorption was the only abnormality reported in control litters. Body weights of fetuses in the treated group were lower than those in the control group **[by 27%]**. The highest rates of malformation occurred in fore- and hindlimbs. Incidences of forepaw poly- or ectrodactyly ranged from 91.7–92.9% after exposure in the light periods and from 11.6–83.2% after exposure in dark

periods. Incidences of hindpaw poly- or ectrodactyly were 50–78.3% with exposure during the light period and 0–68.1% with exposure during the dark period. Retarded forelimbs were observed at incidences of 0–29% during the light period and 0–17.5% in the dark period. Circadian components were found to be highly significant ($P < 0.001$ or $= 0.005$) for fore-and hindlimb malformations. Although the incidence of teratogenesis was greater after exposure during the light period, the maximum amplitude for malformation was associated with exposure during the transition from dark to light (i.e., dawn) ($P < 0.0001$). A significant negative correlation (-0.903, $P < 0.025$) was observed between dam activity level after injection and malformation rates. The study authors hypothesized that higher activity levels may have enhanced clearance of hydroxyurea. The study authors concluded that teratogenesis was greater when hydroxyurea was injected during the light phase.

Strengths/Weaknesses: The use of a single dose level is a weakness. The circadian rhythm idea lacks credibility.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful.

Barr and Beaudoin (1981), supported by the American Cancer Society, examined the role of circadian growth variations in hydroxyurea-induced developmental toxicity in rats. Two rat stocks were tested in parallel experiments. One stock was maintained by the study authors, and the second stock was purchased from an animal breeder. Female Wistar rats were caged overnight with males, and 6 AM on the day of sperm detection was defined as GD 0.0. Beginning at 6 AM of GD 9.0 and continuing at 6-hr intervals until 12 PM of GD 10.75, separate groups of rats were i.p. injected once with hydroxyurea in saline. A dose of 200 mg/kg was administered on GD 9.0, and the dose was increased by 25 mg/kg bw at each 6-hr interval to attain a final dose of 375 mg/kg bw/day on GD 10.75. At each time period, ~8–11 dams/stock were injected with hydroxyurea. For rats obtained from the commercial breeder, ~27 dams were i.p. injected with saline vehicle. Because there was no evidence of effects after injection at different time periods, saline-injected dams from different time periods were combined into one group. There was no control group for the stock maintained in the authors' laboratory. Fetuses were delivered by cesarean section on GD 21. Fetuses and placentas were weighed, and fetuses were examined for external and soft-tissue malformations. Comparisons were made between effects in control and treated groups in the stock from the breeder. Differences of effect in the two stocks were also compared. Statistical analyses included Student's *t*-test, χ^2 test, regression-correlation analysis, and least squares method.

No correlations were found between time of hydroxyurea administration and fetal weight, placental weight, or resorptions. In the stock obtained from the commercial breeder, some significant differences were noted between the control and hydroxyurea-treated groups. In hydroxyurea-treated groups, significant reductions were observed in fetal body weight (mean $\pm$ SE of 4.10 ± 0.06–4.45 ± 0.05 g compared to 4.89 ± 0.02 g in controls) and placental weight (339 ± 5–399 ± 6 g compared to 405 ± 3 g). Whether the resorption rate was significantly increased in the hydroxyurea compared to the control groups (9.4–15.9% compared to 6.1%) was not indicated. The malformation rate was 57.7–97.4% in the hydroxyurea groups and 3.2% in the control group. The most frequently observed malformations included anophthalmia/microphthalmia, hydrocephalus, exencephaly, ear dysplasia, maxillary hypoplasia, protruding tongue, and hydronephrosis. The study authors noted no correlations between time of hydroxyurea administration and total malformations. Some cyclic variations were observed for incidence of hydronephrosis and retention of left umbilical artery, but it was not clear if the effects were due to circadian rhythms. **[The authors' description of cyclic variation effects was difficult to interpret. Based on data presented in Table 2 of the study, it generally appeared that incidences of individual malformation, with the exception of hydronephrosis and retention of left umbilical artery, were greatest when hydroxyurea was administered before GD 9.75, i.e., before 6 PM of GD 9. Levels of statistical significance were often not defined clearly for the effects described above, but in some cases those levels were $P < 0.05$ or 0.01.]**

Some differences were noted between the rats obtained from commercial breeder and those maintained in the authors' laboratory. In the stock maintained at the authors' laboratory, fetal and placental weights were greater, and most types of malformations occurred at a lower incidence. Though hydroxyurea was clearly teratogenic in both stocks, defects commonly observed in the commercial stock but not in the authors' stock included protruding tongue, hindlimb dysplasia, tail malformations, and anal atresia. There were some differences between stocks in peak time of treatment for malformations. The study authors concluded that their study was not able to determine if circadian embryonic growth affects response to teratogens, but they noted that differences were observed in types and timing of malformations in two different rat stocks of the same strain.

Strengths/Weaknesses: This study provided useful information on whether the time of day can influence outcome in single exposure of hydroxyurea. The authors had adequate group sizes and used an appropriate dose range. The study outcome showed that the time of day did not affect total malformation rates of other developmental toxicity endpoints, suggesting this is one variable that should not be considered in other studies with this material. However, this study was not designed to identify hazards associated with repeated exposures on several days of gestation and did not have appropriate concurrent controls for several of the experimental groups. The changing of dose level across different days is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Rajewsky et al. (1971), supported by the German Research Council, examined the effect of transplacental exposure to hydroxyurea on proliferating cells in the embryo. On GD 18, BD IX rats were i.p. dosed with hydroxyurea **[purity not given]** in saline at doses of 250 or 500 mg/kg bw. ^{3}H-methyl thymidine was injected i.p. at different times during a 10-hr period after hydroxyurea exposure; 2–8 embryos were killed 30 min after each ^{3}H-methyl thymidine exposure. Embryos were subjected to autoradiography, and radioactivity levels were measured by liquid scintillation counting in some studies. Hydroxyurea concentrations in embryos and

maternal blood were determined using a colorimetric procedure. In additional experiments, the rate of ^{3}H-methyl thymidine intake into fetus and percent mitotic cells in liver were determined for up to 18 hr after dosing with 250 mg/kg bw hydroxyurea.

^{3}H-methyl thymidine intake was inhibited in embryos for 2.5 hr after exposure to 250 mg/kg bw hydroxyurea and for 4.5 hr after exposure to 500 mg/kg bw hydroxyurea. The hydroxyurea concentration decreased exponentially in embryos, with a half-life of 45 min. This half-life was longer than that reported for maternal blood (20 min). Despite the slower elimination of hydroxyurea from the fetal compartment, the study authors reported that inhibition of DNA synthesis was not longer in fetuses than in dams. **[Dam data were not shown.]** In the time-response study, exposure to hydroxyurea resulted in blocked DNA synthesis for 2.5 hr, a peak of DNA synthesis at 7 hr, and a maximum mitotic index at 7–9 hr. Although difficult to discern at later time periods, mitosis appeared to decrease at ~11–12 hr and peak again at ~15 hr after hydroxyurea exposure. Hepatocyte mitosis peaked at 7–9 hr after hydroxyurea exposure.

Strengths/Weaknesses: Administration of hydroxyurea on GD 18 (during the fetal period) showed effects during a period when the animal should still be susceptible, although administration earlier in gestation may have been preferable. The measured effects on DNA synthesis at two dose levels and multiple times is a strength. The use of direct measures of cell division was useful in proving continued susceptibility. However, the study does not provide the type of information needed for hazard identification, to produce a dose response-curve, or to identify a NOAEL or LOAEL.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Additional information on the effects of hydroxyurea on DNA synthesis in rat embryos is included in the discussion of Scott et al. (1971) in Section 3.2.1.2.

Krowke and Bochert (1975), supported by the German Research Council, examined possible inhibition of RNA synthesis in Wistar rat fetuses exposed to hydroxyurea. In the first experiment, rats **[number treated not specified]** were i.v. injected on GD 12 (GD 0 = day of sperm detection) with 250 mg/kg bw hydroxyurea **[purity not given]**, ^{14}C-glucose, and ^{32}P-phosphate. Incorporation of label into different cell fractions was determined at 45, 90, and 180 min after exposure. In the second experiment, rats were injected **[presumably iv]** with 250, 500, or 750 mg/kg bw hydroxyurea on GD 12. Two hours later, the rats were i.v. injected with ^{14}C-glucose and ^{32}P-phosphate. At least four rats/group were killed 3 hr later, which was 5 hr after dosing with hydroxyurea. **[Control animals were included in both experiments, but treatment of those controls was not specified.]** Radioactivity levels were measured by liquid scintillation counting.

In the time-response study, incorporation of ^{14}C and ^{32}P into DNA was reduced by 50% or more compared to control levels at all time periods. Increases in ^{14}C in the acid-soluble fraction of fetuses 90 min after injection were consistent with increased ^{14}C activity in maternal blood, and the study authors indicated that the effect occurred due to increased availability from the maternal to the fetal compartment. Increased ^{14}C activity in lipid, RNA, and protein fractions at 90 min after exposure led study authors to conclude that hydroxyurea did not affect metabolic processes such as synthesis of those components. In the dose-response study, dose-related reductions in ^{14}C and ^{32}P incorporation into DNA were observed. With the longer exposure to hydroxyurea in the second experiment (i.e., 5 vs. 3 hr), dose-related reductions in ^{32}P incorporation in RNA were observed at hydroxyurea doses ≥250 mg/kg bw. Reductions in RNA ^{14}C levels were observed at hydroxyurea doses ≥500 mg/kg bw. The study authors concluded that hydroxyurea inhibits DNA synthesis in rat embryos at 3 hr after exposure and RNA synthesis at 5 hr after exposure.

Strengths/Weaknesses: Measurement of effects of hydroxyurea on incorporation of radiolabeled substrates over time and across dose is a strength. The study used a dose level in the range of a LOAEL (250 mg/kg) and did not find an effect, possibly due to insensitivity of the methods used. In the follow-up experiments, a very high dose level of 500 or 750 mg/kg was used. However, the amount of cell death and other toxicity from hydroxyurea at these dose levels does not allow for a useful experiment to examine subtle changes with these methods. Use of a single day of gestation is a weakness.

Utility (Adequacy) of CERHR Evaluation Process: This study is of limited utility for the evaluation, although it supports impairment of DNA synthesis as a mechanism of toxicity.

Amortegui et al. (1983), support not indicated, examined the effect of hydroxyurea exposure on fetal growth in rats. The effects of cycloheximide were also examined but will not be discussed here. Ten Sprague-Dawley rats/group were injected i.p. with saline vehicle or 1800 mg/kg bw hydroxyurea on GD 15. **[Hydroxyurea purity was not indicated. It was not clear how authors defined the day of vaginal sperm or plug.]** Dams were killed on GD 21. Three fetuses/dam from midline locations in the uterus were weighed and killed. Placenta and fetal brain, liver, and heart were weighed. One fetus/litter was examined histologically, but it does not appear that data were presented. DNA, RNA, protein, and glycogen levels were measured in heart, liver, brain, and kidney. Data were analyzed by nested ANOVA. **[The authors stated that a second experiment was conducted in which dams were allowed to deliver pups, and pups were examined at 28 days of age. The Methods sections indicated that biochemical endpoints in pup organs were measured at 28 days of age, but according to tables and figures in the Results section, both organ weights and biochemical endpoints were measured in fetuses. In this study summary, it is assumed that the Results section is correct, and data were reported for fetuses.]**

Body weight of dams was not affected by hydroxyurea treatment. **[Data were not shown.]** Body weight of fetuses in the hydroxyurea group was reduced significantly to ~70% of control levels. Results of organ weight and biochemical analyses are summarized in Table 40. Weights were decreased for brain, kidney, and liver, and the decreases in weights were accompanied by reduced levels of protein, RNA, DNA, or carbohydrates. There were no decreases in heart or placenta weight, but there were some changes in biochemical endpoints. The study authors concluded that hydroxyurea induced profound changes in organ weights and biochemical composition

Table 40
Organ Weight and Biochemical Findings in Rat Fetuses Prenatally Exposed to Hydroxyurea

Organ	Weight effect[a]	Biochemical findings
Brain	↓25%	↓ Protein, RNA, DNA
Kidney	↓55%	↓ RNA, DNA, carbohydrate
Heart	↔	↑ DNA
Liver	↓28%	↓ Protein, RNA, DNA
Placenta	↔	↓ Protein, RNA

From Amortegui and Coyne (1983).
[a]Values estimated by CERHR from a graph.
↑,↓, Statistically significant increase or decrease compared to controls; ↔, no statistically significant effects compared to controls.

in fetal rats at doses that had no apparent effect on dam body weight or behavior.

Strengths/Weaknesses: This study examined several endpoints that provide an overall indication of growth retardation after hydroxyurea exposure. The measurement of organ weights and macromolecular content is a strength. Weaknesses are the use of an extremely high dose level and the difference in time between when the dose was administered and when the endpoints were measured, allowing for significant recovery.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Sugrue and DeSesso (1982), supported in part by the Orthopedic Research and Education Foundation, examined the effect of hydroxyurea exposure on glycosaminoglycan composition of fetal rat forelimb buds. On GD 12 or 13 (day of vaginal sperm = GD 0), Wistar rats were i.p. injected with saline vehicle or 1000 mg/kg bw hydroxyurea **[purity not given]**. Dams were killed on GD 20, and fetuses were prepared for evaluation of skeleton and cartilage. Additional dams were killed at 12-hr intervals between 3 and 96 hr after hydroxyurea treatment for histologic and histochemical examinations of fetal limb buds. Glycosaminoglycans were isolated and separated by electrophoresis. Digestion by specific polysaccharidase was used to confirm identification of each glycosaminoglycan. At each time period, 7–10 fetuses/litter were pooled. Mitotic index was determined in five limbs from five embryos. Data were analyzed by linear regression and Student's *t*-test.

Examination of GD 20 fetuses that were exposed to hydroxyurea on GD 12 showed forelimb malformations in 72% of the fetuses, and ectrodactyly was observed in 66% of those fetuses. In GD 20 fetuses that were exposed to hydroxyurea on GD 13, hindlimb and distal forelimb malformations were observed; all forelimbs had defects, and 67% of forelimbs lacked one or more digits. Histologic evaluation of fetuses treated on GD 12 or 13 showed cell death (e.g., cell debris and pyknotic nuclei) in limb-bud mesenchyme within 3 hr of exposure. Intercellular spaces were enlarged at 12 hr after treatment on GD 12. At 12–60 hr after treatment of GD 12 fetuses and 24–36 hr after treatment of GD 13 fetuses, hyaluronic acid levels in forelimbs exceeded levels observed in control fetuses. Chondroitin sulfate levels were decreased from 48–72 hr after treatment on GD 12, and at 36–48 hr after treatment on GD 13. Histolocalization analysis showed positive staining for hyaluronic-rich material beneath the basement membrane of the peripheral region and sub-ridge zone of limb-buds at 24 hr after treatment on GD 12. A similar increase in hyaluronic acid was observed at 12–24 hr after treatment on GD 13, but the increase in the peripheral zone was not as extensive as in fetuses treated on GD 12. Glycosaminoglycans in the central region were not affected by hydroxyurea treatment on either day. Mitotic activity was reduced in the sub-ridge and peripheral regions at 12–24 hr but increased above control levels at 36 hr after hydroxyurea treatment on GD 12. After treatment on GD 13, mitotic activity was decreased in the sub-ridge zone at 12–24 hr but was increased at 36–48 hr after treatment. The study authors concluded that increases in hyaluronic acid levels and mitosis represented repair mechanisms.

Strengths/Weaknesses: A strength of this study is the following of pathogenesis of limb malformations from the time of dose, including the use of histology, glycosaminoglycan composition, and mitotic index. This study suggested that repair to the limb bud can occur after insult from hydroxyurea. The investigation was conducted very thoroughly, was carried out with good techniques, and provides a plausible mechanism of action of hydroxyurea teratogenicity, at least for limb bud effects. Weaknesses are that use of a single high dose of hydroxyurea on a single day of gestation limits utility for determining a NOAEL or LOAEL, and the glycosaminoglycan data were not very useful.

Utility (Adequacy) of CERHR Evaluation Process: This study is of limited utility.

Teramoto et al. (1980), support not indicated, examined the developmental toxicity of hydroxyurea after intra-amniotic injection of rats. The main focus of the study was ethylene thiourea, which will not be discussed here. On GD 12 (GD 0 = day of vaginal sperm), the uteri of female Wistar-Imamichi rats were exposed and each amniotic sac of one horn was injected with hydroxyurea **[purity not given]** at 500, 1000, or 2000 μg in 10 μL distilled water. The amniotic sacs of the other uterine horn were injected with 10 μL distilled water vehicle. A total of 10–17 dams/group were exposed to hydroxyurea, and all dams (n = 38) were exposed to the distilled water vehicle. Dams were killed on GD 20, and implantation sites were examined for resorbed fetuses. Live fetuses were weighed and assessed for external and visceral abnormalities. Data were analyzed by Student's *t*-test or Fisher exact test.

The resorption rate in the distilled water control (44%) was reported to be higher than historical control values from the study authors' laboratory (5–15%). Resorption incidence was further increased in each hydroxyurea treatment group (89%, 98%, and 96% in the low-, mid-, and high-dose groups). The study authors reported reductions in fetal survival and growth, but statistical significance was only reported for decreased body weight in females from the low-dose group. **[Compared to controls, body weights in low-, mid, and high-dose group were reduced by 14, 55, and 24% in males and 14, 28, and 41% in females.]** The malformation rate was increased in each dose group and was reported at 6% in controls, 56% in the low-dose group, and 100% in the mid- and high-dose groups. The types of malformations most commonly observed with hydroxyurea exposure included cleft palate, micrognathia, oligodactyly, syndactyly, club foot, short or kinky tail, anal atresia, and

hydronephrosis. Based on their findings, the study authors concluded that hydroxyurea induced malformations as a result of direct action on rat embryos.

Strengths/Weaknesses: The use of multiple dose levels is a strength. The intra-amniotic route of administration is a weakness. Because hydroxyurea can cross the placenta from the maternal to the fetal compartment, it is also possible that it can cross from the conceptus to the maternal unit. Therefore, this study did not eliminate effects mediated from the maternal component as claimed.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful.

Salaman and Birkett (1974), supported by the Nuffield Foundation, examined the effect of hydroxyurea exposure on androgen-induced sexual differentiation in rats. The effects of other compounds were also examined but will not be discussed here. Four-day-old female Wistar rats were s.c. injected with testosterone propionate in oil alone or in combination with hydroxyurea **[purity not given]**. Hydroxyurea was administered in divided doses, together with and 6 hr after exposure to testosterone propionate. The doses used in this study were 30 μg testosterone propionate + 800 mg/kg bw hydroxyurea, 80 μg testosterone propionate + 500 mg/kg bw hydroxyurea, and 200 μg testosterone propionate + 400 mg/kg bw hydroxyurea. For each dosing scenario described above, a negative control was used and rats were exposed to the same doses of testosterone propionate alone. Exposure to 400 mg/kg bw hydroxyurea alone was also examined. The number of rats examined in each dose group was 7–21. Vaginal smears were assessed daily for at least 3 weeks between the ages of 80–110 days. Animals were considered to be acyclic if they displayed eight or more consecutive cornified vaginal smears. Ovaries were removed, on the day of estrous if possible, and weighed and examined for recent corpora lutea and presence of eggs in the oviduct. Statistical analyses included Fisher exact test and Mann-Whitney *U*-test.

In control animals observed under each exposure scenario, 0–6% were acyclic, 94–100% had recent corpora lutea, and 81–93% had ova in the oviducts. Exposure to testosterone propionate adversely affected each of these endpoints in a dose-related manner. In the testosterone propionate groups, 70–100% of animals were acyclic, 0–20% had recent corpora lutea, and 0–10% had ova in oviducts. Co-exposure to hydroxyurea protected the rats against the masculinization effects of testosterone. In the rats co-exposed to testosterone propionate and hydroxyurea, 18–31% were acyclic, 69–86% had recent corpora lutea, and 43–55% had ova in oviducts. Compared to the group exposed to testosterone propionate alone, statistical significance ($P < 0.01$ or 0.001) was obtained for all endpoints in the groups exposed to testosterone propionate and hydroxyurea, with the exception of percent acyclic animals and animals with ova in oviduct in the group co-exposed to 30 μg testosterone propionate and 800 mg/kg bw hydroxyurea. Exposure to 400 mg/kg bw/day hydroxyurea alone had no significant effects compared to the negative control group for any endpoint examined. Rats exposed to testosterone propionate alone had lower ovarian weights than negative controls, but ovarian weights were not significantly different from control values after co-exposure of testosterone propionate and hydroxyurea. The study authors concluded that although the exact method of sexual differentiation is not known, DNA synthesis is most likely involved.

Strengths/Weaknesses: The co-treatment with testosterone of hydroxyurea-exposed animals is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful.

3.2.1.4 Parenteral exposure—prenatal developmental toxicity with co-exposure to other compounds or conditions: This section addresses studies involving prenatal developmental toxicity after co-exposure to hydroxyurea and certain conditions or chemicals. Co-exposure factors included:

- DNA precursors;
- Chemical agents; and
- Malnutrition.

Chaube and Murphy (1973), supported in part by the American Cancer Society; Albert and Mary Lasker Foundation; NIH; US Department of Health, Education, and Welfare; and the Association for the Aid of Crippled Children, examined the effect of DNA precursors on hydroxyurea-induced malformations in rats. A series of studies were conducted in which Wistar rats were i.p. injected with 500 mg/kg bw hydroxyurea **[purity not given]** in saline vehicle, pyrimidines, and or a combination of 500 mg/kg bw hydroxyurea and pyrimidines on GD 11 (GD 0 = day of sperm detection). The different exposure conditions for pyrimidines are summarized in Table 41. There were 2–19 dams in each treatment group. Animals were killed on GD 21. Implantation sites were assessed, and fetuses were examined for viability, body weight, and gross malformations. Skeletal malformations were examined in some pups from each litter.

In these studies, no malformations or increases in mortality were observed in rats exposed to pyrimidines, but the malformation rate was ~99% after exposure to hydroxyurea alone. The types of malformation observed with hydroxyurea exposure were cleft palate, micrognathia, defects of foreleg and hind leg, syndactylous forepaw, syn- or polydactylous hindpaw, and short kinked tail. Exposure to hydroxyurea alone did not increase mortality rate. Table 41 lists the experimental exposure conditions and results for rats co-exposed to pyrimidines and hydroxyurea. As summarized in Table 41, some doses of pyrimidine compounds protected against hydroxyurea-induced malformations. At higher doses, some pyrimidines increased fetal mortality on co-administration with hydroxyurea. The study authors concluded that deoxycytidylic acid was the cytidine compound with the greatest protective effect and that protective effects were greater for cytidine than for uridine derivatives.

Strengths/Weaknesses: The authors tested an interesting concept in this mechanistic study. The use of a potentially protective agent (DNA precursors) was based on a proposed mechanism of action of inhibiting DNA synthesis through inhibition of the conversion of ribonucleotides to deoxyribonucleotides. The endpoints evaluated were relatively simple, straightforward, and not open to a lot of bias. However, the study used a single, relatively high dose level on 1 single day of

Table 41
Effects of Pyrimidines on Developmental Toxicity Induced by 500 mg/kg bw Hydroxyurea Intraperitoneally on GD 11 in Rats

Exposure condition[a]	Major findings
Simultaneous i.p. injection with hydroxyurea and 3.7–1000 mg/kg bw deoxycytidylic acid	No abnormal fetuses observed when 700 mg/kg bw deoxycytidylic acid was administered with hydroxyurea; dose-related reductions in fetal abnormalities were observed when 3.7–500 mg/kg bw deoxycytidylic acid was administered with hydroxyurea; 1000 mg/kg bw deoxycytidylic acid did not protect fetuses from hydroxyurea effects and increased mortality (14.6% mortality rate).
700 mg/kg bw deoxycytidylic acid administered 15–150 min before and after hydroxyurea treatment	When deoxycytidylic acid was administered before hydroxyurea, protection against fetal malformations was greatest when it was administered 15 min before hydroxyurea treatment (6.8% abnormal fetuses); protection was reduced with increased interval between deoxycytidylic acid treatment and hydroxyurea exposure. When deoxycytidylic acid was administered after hydroxyurea, 37.5% abnormal fetuses were observed with its administration 15 min after hydroxyurea treatment; its protective effects were decreased with increased delay of deoxycytidylic acid treatment after hydroxyurea exposure. Fetal mortality was increased with administration of deoxycytidylic acid ≥60 min after hydroxyurea injection.
Rats injected with a solution containing 500 mg/kg bw hydroxyurea and 700 mg/kg bw deoxycytidylic acid that was left at room temperature for 15–120 min	Protective effects of deoxycytidylic acid declined when solution was stored at room temperature for 15 minutes or more (0% abnormal fetuses at time 0 and ~15% abnormal fetuses at 15–120 min after solution preparation).
Rats injected with hydroxyurea and 7.5–1500 mg/kg bw cytidine, cytidylic acid, or deoxycytidine	Some protective effects were observed for each compound, with maximum protective effects against hydroxyurea-induced malformations at 250–700 mg/kg bw cytidine (~25–27% abnormal fetuses), 31 mg/kg bw cytidylic acid (50% abnormal fetuses), and 62–125 mg/kg bw deoxycytidine (66.2% abnormal fetuses). Fetal mortality was increased with co-administration of hydroxyurea and ≥250 mg/kg bw cytidine (≥15.8% mortality), ≥62 mg/kg bw cytidylic acid (33% mortality), and ≥700 mg/kg bw deoxycytidine (≥12.2% mortality).
Rats were injected with hydroxyurea and 250–1000 mg/kg bw uridine, uridylic acid, deoxyuridine, or deoxyuridylic acid	Maximum protective effects against hydroxyurea-induced malformations were observed at 700 mg/kg bw uridine (30.6% abnormal fetuses), 250–500 mg/kg bw uridylic acid (69.2% abnormal fetuses), and 250 mg/kg bw deoxyuridylic acid (52.4% abnormal fetuses); no protection was observed with deoxyuridine treatment. Fetal mortality was increased with co-administration of hydroxyurea and ≥700 mg/kg bw uridine (≥36% mortality), 700 mg/kg bw uridylic acid (33% mortality), all doses of deoxyuridine (≥26.2% mortality), and all doses of deoxyuridylic acid (≥16% mortality).
Rats were injected with hydroxyurea and 250–700 mg/kg bw thymidine, or thymidylic acid	No protective effects were observed.

From Chaube and Murphy (1973).
[a]The hydroxyurea dose in all the studies was 500 mg/kg bw (mg/kg bw) administered by intraperitoneal (i.p.) injection on gestational day (GD) 11; additional groups of rats were treated with 500 mg/kg bw hydroxyurea alone or pyrimidines alone in all studies.

gestation, and therefore it is not clear if similar mechanisms occur on other days of gestation or at lower doses of hydroxyurea. Although this is an interesting study, it has limited utility for this evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is of utility in supporting inhibition of DNA synthesis as a mechanism of toxicity. It has little utility for a quantitative assessment of developmental toxicity.

Ritter (1984), supported by NIH, examined possible potentiation of hydroxyurea-induced embryotoxicity in rats by agents that inhibit synthesis of DNA, RNA, protein, or purines. Wistar rats were i.p. injected with hydroxyurea **[purity not given]** in distilled water at doses of 500 mg/kg bw on GD 12 (n = 17/group; GD 0 = the morning sperm were found) or 300 mg/kg bw on GD 10 (n = 13/group). A concurrent control group consisted of 12 untreated rats. Additional groups of 7–10 rats were co-administered hydroxyurea and 5-fluoro-2′-deoxyuridine, cytosine arabinoside, hadacidin, actinomycin D, cycloheximide, or emetine. Dams were killed on GD 20. Implantation sites were examined, and fetuses were weighed and evaluated for external and

Table 42
Malformation Rates for Hydroxyurea and Compounds That Potentiated the Malformation Rate

Treatment (mg/kg bw)	% Malformations in live fetuses (mean ± SE)
GD 12	
500 Hydroxyurea	9.6 ± 3.2
50 Arabinoside	7.3 ± 4.6
500 Hydroxyurea + 50 arabinoside	50.4 ± 13.1
1000 Hadacidin	29.0 ± 13.2
1000 Hadacidin + 500 hydroxyurea	98.5 ± 1.0
0.5 Cycloheximide	17.2 ± 7.1
0.5 Cycloheximide + 500 hydroxyurea	86.0 ± 10.4
5 Emitine	1.2 ± 1.2
5 Emitine + 500 hydroxyurea	21.5 ± 10.1
GD 10	
300 Hydroxyurea	20.5 ± 5.6
50 Arabinoside	4.6 ± 2.4
300 Hydroxyurea + 50 arabinoside	78.3 ± 5.6
0.2 Actinomycin D	6.6 ± 3.1
300 Hydroxyurea + 0.2 actinomycin D	80.0 ± 9.0

From Ritter (1984).
GD, gestational day.

skeletal malformations. The litter was considered the experimental unit in statistical analyses conducted by χ^2 one-sample test.

Treatment with hydroxyurea did not seem to increase the resorption rate, which was reported at ~7.8% in the control group. The only significant potentiation of resorption rate involving hydroxyurea occurred with hadacidin co-administration on GD 10. Percent resorption rates ± SE were 9.2 ± 4.6% for 300 mg/kg hydroxyurea, 7.6 ± 5.5% for 2000 mg/kg bw hadacidin, and 95.6 ± 2.9% for the two compounds combined. No malformations were observed in control groups. Treatment with 500 mg/kg bw hydroxyurea on GD 12 or 300 mg/kg bw on GD 10 increased the malformation rate (Table 42). The types of malformations observed with treatment on GD 12 were diaphragmatic hernia, clubbed hindlimb, kinky tail, heart defects, hydronephrosis, hydroureter, and micrognathia. Malformations observed with treatment on GD 10 included anophthalmia, folded retina, fused ribs, heart defects, hydrocephalus, kinky tail, and microphthalmia. The malformation rate was significantly increased when hydroxyurea was administered with arabinoside, hadacidin, cycloheximide, emitine, and actinomycin D. Table 42 summarizes malformation rates observed with each compound alone and in combination with hydroxyurea. The study authors concluded that combination of a wide variety of metabolic inhibitors affecting different biochemical pathways potentiated embryotoxicity.

Strengths/Weaknesses: The issue of potentiation of teratogenic effects after hydroxyurea exposure is important in that patients may be receiving several pharmaceutical agents simultaneously. The author used adequate group size to investigate this reasonable hypothesis. The weakness of this study is the use of very high doses of hydroxyurea on single days of gestation and therefore, few conclusions can be drawn from this study. Most inhibitors potentiated the effects of hydroxyurea; there was little specificity.

Table 43
Developmental Toxicity Effects in Rats Treated with Hydroxyurea Alone or in Combination with Caffeine

Treatment (mg/kg bw/day)	Fetal weight	Resorptions	Malformed live fetuses
500 Hydroxyurea	↓12%	↑2.5-fold	↑14-fold
75 Caffeine	↑2.5%	↑2.5-fold	↑25-fold
150 Caffeine	↓3.3%	↑3.2-fold	↑5-fold
500 Hydroxyurea + 75 caffeine	↓27%	↑3.6-fold	↑10-fold
500 Hydroxyurea + 150 caffeine	↓47%	↑22-fold	↑190-fold

From Ritter et al. (1982).
↑,↓, Increase or decrease, respectively, compared to historical control values. **[It was difficult to determine statistically significant differences between treatment groups using the authors' description.]**

Utility (Adequacy) of CERHR Evaluation Process: This study has limited utility.

Ritter et al. (1982), supported by NIH, examined the developmental toxicity in rats of hydroxyurea alone or in combination with caffeine. On GD 12 (GD 0 = day of vaginal sperm), Wistar rats were i.p. injected with 500 mg/kg bw hydroxyurea **[purity not given]** in distilled water alone or in combination with caffeine at 75 or 150 mg/kg bw. Rats were also exposed to each caffeine dose alone. Doses were selected to result in definite but low malformations rates. **[The number of dams treated was not reported. There was apparently no concurrent control, and values were compared to historic controls.]** Pregnancies were terminated on GD 20 for examination of implantation sites and external and skeletal malformations. Data were presented as litter averages. Statistical analyses included ANOVA and Newman-Keuls range test.

Study results are summarized in Table 43. Exposure to hydroxyurea resulted in increased resorptions and malformed fetuses. The types of malformations observed included diaphragmatic hernia, heart defects, kinky tail, and micrognathia. Co-administration with caffeine resulted in a dose-related increase in the resorption rate and malformation incidence. The study authors concluded that the embryotoxicity of hydroxyurea was potentiated by caffeine at doses greatly exceeding typical human intake of caffeine, which were estimated to be 3–30 mg/kg bw.

Strengths/Weaknesses: The issue of potentiation of teratogenic effects after hydroxyurea exposure with co-exposure to caffeine is interesting given the real possibility of these exposures occurring. The strengths of this investigation are the adequate group sizes and the use of other inhibitors in determining outcome. Weaknesses of this study are the use of a single high dose on a single day of gestation (people would be exposed to lower doses over several days/weeks of gestation) and lack of concurrent controls.

Utility (Adequacy) of CERHR Process: This study has limited utility.

Chahoud et al. (2002), supported by the Brazilian National Research Council, examined the effects of malnutrition on hydroxyurea-induced developmental toxicity in rats. The effects of cyclophosphamide were also examined but will not be discussed here. On GD 0

[presumably the day of vaginal sperm or plug], Wistar rats were assigned to groups fed normal diet (24% protein, 56% starch) or a protein-energy deficient diet (8% protein, 18.4% starch). The normal diet was provided ad lib, whereas intake of the protein-energy-deficient diet was limited to 20 g/day. On GD 11, rats from both dietary groups were i.p. injected with distilled water or hydroxyurea **[purity not reported]** at 300 or 500 mg/kg bw. There were 15–17 dams/group that were fed the normal diet. Among the malnourished groups, there were 17 dams in the control group and seven dams/group treated with hydroxyurea. Dams were killed on GD 21, implantation sites were examined, and fetuses were assessed for viability. Live fetuses were weighed and examined for external and skeletal defects. Data for maternal weight gain were analyzed by Kruskal-Wallis test followed by Mann-Whitney *U*-test. All other data were analyzed by ANOVA and Student's *t*-test.

In rats fed the normal diet group and exposed to the high dose of hydroxyurea, maternal weight, weight gain during gestation, and gravid uterus weight were significantly lower than controls in the same dietary group. In the malnourished group, significant differences observed in rats exposed to the high hydroxyurea dose compared to controls included lower weight gain and gravid uterus weight. Maternal weight, weight gain, and gravid uterus weight were significantly lower in malnourished than normal-diet rats in the control and both hydroxyurea groups. Nether hydroxyurea dose affected the number of implantation sites. At the high hydroxyurea dose, the percentage of resorbed implantation sites was increased in the normal dietary group (35 vs. 5% in controls) and the malnourished group (32 vs. 6%) and percent live fetuses was reduced in the normal diet group (65 vs. 95%) and the malnourished group (68 vs. 94%). **[Modeling resorptions/litter in the well-nourished group, BMD_{10} = 166 mg/kg bw, $BMDL_{10}$ = 93 mg/kg bw, BMD_{1SD} = 342 mg/kg bw, and $BMDL_{1SD}$ = 272 mg/kg bw.]** Diet regimen did not affect either endpoint. Fetal body weights at the high hydroxyurea dose compared to the control group were decreased in the normal diet group **[by ~24%]** and the malnourished group **[by ~20%]**. Fetal body weights in malnourished group compared to the normal diet group were significantly lower in the control and both treatment groups. **[Modeling fetal body weight in the well-nourished group, BMD_{10} = 363 mg/kg bw, $BMDL_{10}$ = 293 mg/kg bw, BMD_{1SD} = 328 mg/kg bw, and $BMDL_{1SD}$ = 244 mg/kg bw.]**

In rats from the normal dietary group, abnormalities of thoracic centra were significantly higher in the low-dose than in the control group (100 vs. 5.9% of litters affected **[BMD_{10} = 17 mg/kg bw, $BMDL_{10}$ = 7 mg/kg bw]**). Numerous malformations were increased in the high-dose compared to control rats in the normal diet group, and incidences of significant malformations ranged from 26.7–100% of litters affected in the high-dose group compared to 0–52.9% of litters affected in the control group. In the high-dose hydroxyurea group, malformations affected skull, forelimbs, hindlimbs, sternum, thorax, and vertebral column. **[Modeling the malformations seen most commonly on a litter basis: for cleft palate, BMD_{10} = 454 mg/kg bw and $BMDL_{10}$ = 320 mg/kg bw; for absent forelimb digit 5, BMD_{10} = 434 mg/kg bw and $BMDL_{10}$ = 312 mg/kg bw; and for malpositioned hindlimb, BMD_{10} = 412 mg/kg bw and $BMDL_{10}$ = 308 mg/kg bw.]** In the malnourished group, one type of skull malformation and two types of vertebral column malformations were observed at an increased incidence in the low-dose group compared to the control group; litter incidences were 71.4–85.7% in the low dose group and 0–52.9% in the control group. Numerous skeletal malformations were increased in malnourished rats from the high-dose compared to the control group, with litter incidences for significant effects ranging from 28.6–100% in the high-dose group and from 0–52.9% in the control group. Some malformations in the sternum occurred at a higher incidence in malnourished controls than in normal controls, but the study authors noted that the values were close to historic control rates. The study authors noted that with the exception of sternum malformations, the protein-energy deficient diet attenuated some malformations induced by hydroxyurea treatment. Examples included observation of cleft palate and gastroschisis only in the normal diet group and decreased incidences of some skull, hindpaw, and vertebrae malformations in malnourished compared to normal diet rats exposed to hydroxyurea. The study authors concluded that malnutrition had no effect on embryo lethality, did contribute to weight gain reductions, and did attenuate teratogenicity.

Strengths/Weaknesses: The possibility that altered nutritional status can affect the malformation type/incidence is an interesting hypothesis as a factor contributing to the developmental outcome after hydroxyurea exposure. Such a mechanism may also be clinically relevant. The authors described very well the maternal toxicity observed after the protein-energy restriction diet and effects on hydroxyurea-induced teratogenicity. However, the use of a single high-dose level of hydroxyurea on a single day of gestation limits the usefulness of the data in predicting effects that may occur at lower dose levels over several days of gestation.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited utility.

3.2.1.5 Parenteral exposure studies examining postnatal developmental toxicity: The following section addresses postnatal developmental toxicity in rats exposed parenterally to hydroxyurea during prenatal or postnatal development. Under each major topic of developmental toxicity, the studies are presented by year of publication with multiple dose studies presented before single-dose studies. The major topics of postnatal developmental toxicity are:

- General development (body weight, survival, malformations);
- Neurobehavior;
- Male reproductive endpoints;
- Female reproductive endpoints;
- Hematologic endpoints; and
- Mechanistic endpoints.

Theisen (1979), supported by the University of Cincinnati, the Hoffmann-LaRoche Foundation, and the Minnesota Medical Foundation, examined the effect of hydroxyurea exposure on DNA synthesis in developing nervous tissue of rats. On selected days between GD 12–17 (GD 0 = day of vaginal sperm) Wistar rat dams were

Table 44
Developmental Toxicity in Rats Exposed to Hydroxyurea During Prenatal Development

		% Offspring affected at each dose	
Exposure day	Dose (mg/kg bw)	Malformed	Dead within 26 hr of birth
GD 12	1000	All offspring resorbed	
GD 12.5	750, 1000	20.0, 97.0	42.5, 90.0
GD 13	1000	100.0	9.3
GD 13.5	500, 1000	0, 100.0	0, 6.3
GD 14.0	500, 1000, 1500	0, 12.6, 100.0	0, 1.0, 100.0
GD 14.5	1500	0	10.4
GD 15	1500	0	7.3
GD 16	2000	0	12.8
GD 17	2000	0	3.4
Control	0	0	2.8

From Theisen (1979).
GD, gestational day.

i.p. injected with 500–2000 mg/kg bw hydroxyurea **[purity not given]** in distilled water or aqueous ^{3}H-thymidine.

In one part of the study, dams were killed 1–5 hr after exposure to 1000 mg/kg bw hydroxyurea and ^{3}H-thymidine on GD 12. Embryos were sectioned and subjected to autoradiography to examine labeling in cells of the dorsal root ganglia. Necrotic cells were observed at 3 hr post-dose, and in most dorsal root ganglia, the severity of necrosis was greatest in rostral and caudal poles. **[The numbers of dams exposed and offspring examined were not clear.]**

In another part of the study, one to seven dams in each hydroxyurea group and nine controls were allowed to deliver their litters. Hydroxyurea doses and time periods of exposure for those dams are summarized in Table 44. Within 2–6 hr after birth, offspring were weighed and examined for malformations. Litters were culled to 10 pups. Postnatal growth and maturation were assessed until offspring were killed at 60–70 days of age. At that time, upper lumbar dorsal root ganglia and adjacent spinal cord segments were examined and sectioned. Autoradiography was conducted to examine labeling of cells in the dorsal root ganglia and numbers and size distribution of neurons. Generally, three to seven offspring from three to four litters were examined for each dose and time period. The autoradiography studies were conducted in animals exposed to 500 or 1000 mg/kg bw/day hydroxyurea on GD 13, 13.5, or 14.5.

The study authors noted that parturition time seemed to be normal in dams treated with hydroxyurea, with all deliveries occurring on GD 21 or 22. Percentages of malformed and dying offspring in each hydroxyurea group are summarized in Table 44. Malformations were not observed after exposure to hydroxyurea 500 mg/kg bw/day on GD 13.5 or 14 or to hydroxyurea 1500–2000 mg/kg bw/day administered at GD 15.5 or later. At earlier gestation periods, malformations were increased with exposure to $\geq$750 mg/kg bw hydroxyurea. Susceptibility to embryo lethality was increased at earlier gestation periods. Types of malformations observed (day of exposure) included diaphragmatic hernia and cleft plate (GD 12.5) and ectro- or syndactyly (GD 12.5–14). Pup body weights at birth were significantly ($P<0.05$) reduced at all doses and time periods of exposure (Table 44), with the exception of 500 mg/kg bw hydroxyurea administered on GD 13 or 14. The growth retardation was reported to persist during the postnatal period. No effects were observed for sex ratio. Litter size was reduced only in groups exposed to hydroxyurea 1000 mg/kg bw on GD 12 or 12.5. A hopping gait developed in some animals exposed to hydroxyurea on GD 13 or 14, and the gait appeared to be correlated with spinal cord defects, such as disorganized corticospinal tracts, small dorsal funiculi, shrunken substantia gelatinosa, and increased thickness of the dorsal gray commissure. The gait abnormality was not related to limb malformations.

In adult offspring exposed to 1000 mg/kg bw/day hydroxyurea on GD 13 or 13.5, lumbar dorsal root ganglia were smaller than in controls. Reduced ganglia size was observed in ~20% of animals exposed to 1000 mg/kg bw/day on GD 14. No effects were observed after treatment at later time periods or with 500 mg/kg bw/day hydroxyurea on GD 13.5 or 14 or 750 mg/kg bw/day on GD 12.5. Significant ($P<0.005$, or 0.025) decreases in neuronal numbers in second lumbar ganglia were observed in adult offspring exposed to 1000 mg/kg bw/day hydroxyurea on GD 13 (decreased 62% from controls), GD 13.5 (decreased 48%), or GD 14 (decreased 21%). Exposure to hydroxyurea on GD 13 or 13.5 altered the proportions of large, medium, and small sensory neurons, particularly with exposure on GD 13, which resulted in absence of medium-sized neurons. The study authors concluded, "the magnitude and selectivity of neuronal deficiencies produced by [hydroxyurea] are remarkable and indicative of critical events occurring during terminal cell cycles."

Strengths/Weaknesses: The use of hydroxyurea to affect a specific cell population during the period of (theoretically) greatest susceptibility provides useful information on a possible mode of action for malformations. The authors used multiple dose levels of hydroxyurea and time points for evaluation and investigated a functional outcome. However, the dose range used was very high, exposure was limited to a single day of gestation, and only a small number of samples were evaluated.

Utility (Adequacy) for CERHR Evaluation Process: Although this study is interesting in explaining a

Table 45
Postnatal Developmental Effects in Sprague-Dawley Rats Exposed to Hydroxyurea During Prenatal Development

	Dose (mg/kg bw/day)[a]					
Endpoint	100	200	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Male pup weight at birth	↔	↓8%	126	154	244	141
Female pup weight at birth	↔	↓10%	201	131	198	123
Viability index on PND 4	↔	↓(87.6 vs. 100% in controls)	—	—	—	—
Male pup weight at PND 21	↔	↓8%	227	155	228	150
Female pup weight at PND 21	↔	↓9%	207	137	205	129
Malformed male pups on PND 21	↔	↑(52.9 vs. 0% in controls)	128	107	—	—
Malformed female pups on PND 21	↔	↑(42.5 vs. 0% in controls)	133	111	—	

From Asano and Okaniwa (1987).
↑,↓, Statistically significant increase or decrease, respectively, compared to controls; ↔, no significant effects compared to controls.
[a]No benchmark doses were estimated because the number of litters evaluated could not be determined.
PND, postnatal day.

possible mode-of-action, extrapolation to longer exposure periods is difficult, and the study has limited utility for a quantitative evaluation. The study is useful in showing neurotoxicity.

Asano and Okaniwa (1987), support not indicated, examined developmental toxicity of hydroxyurea in rats. Effects on prenatal development are described in Section 3.2.1.2; effects on postnatal development are described here. On GD 9–12 (GD 0 = day of vaginal sperm), 12–22 Sprague-Dawley rats/group were i.p. injected with 0 (saline vehicle), 100 or 200 mg/kg bw hydroxyurea **[purity not reported]**. Dams were allowed to deliver and nurse litters. Litters were culled to four pups/sex 4 days after delivery. Culled pups were examined for malformations. Dams were killed 21 days after delivery, and implantation sites were examined. Live pups were weighed at birth and at 21 days of age. Surviving pups were killed and necropsied at 21 days of age. There were no significant differences in delivery index, number of implantations, or stillbirth. Results of postnatal evaluations are summarized in Table 45. Exposure to 200 mg/kg bw/day hydroxyurea decreased pup body weight significantly at birth and on PND 21 and reduced pup viability on PND 4 and 21. Malformations were significantly increased in the high-dose group on PND 21 but not PND 4. The types of malformations observed most frequently on PND 21 included hydrocephalus, anophthalmia, and microphthalmia. Statistical analyses included Kruskal-Wallis nonparametric one-way ANOVA, one-way ANOVA, or Wilcoxon rank test.

When malformation rates of different age groups of Sprague-Dawley rats were compared, some significant differences were noted. Incidence of total malformations, lateral ventricle dilatation, microphthalmia, and ventricle septal defect were lower in 4-day-old pups than in fetuses. Incidences of anophthalmia and brain malformations were significantly higher in 21-day-old than in 4-day-old pups. Ventricular septal defects were only observed in fetuses. The study authors stated that higher malformation rates in fetuses than in 4-day-old pups probably resulted from increased perinatal mortality in severely malformed pups and disappearance of some ventricular septal defects over time. According to the study authors, the higher rate of CNS malformations in 21-day-old pups than in 4-day-old pups most likely resulted from latent expression of malformations due to increasing severity with age. The study authors concluded that prenatal exposure of rats to 200 mg/kg bw/day resulted in growth retardation, pup mortality, and malformations.

Strengths/Weaknesses: Observing the incidence of visceral malformations during the postnatal period after prenatal exposures was a useful exercise for hydroxyurea because many of the malformations that are observed are potentially lethal to newborn animals. In addition, observing the difference in the incidence of malformations that occurred on PND 4 versus PND 21 was useful in determining the consequences of these defects. However, there were obvious litter effects that were not corrected for, and the prenatal dosing period was relatively short and did not include the entire period of embryogenesis or the fetal period. The use of uneven group sizes affected the power for detecting an effect, so the incidence at a specific dose level on a specific day to detect a certain malformation was not similar between all the groups.

Utility (Adequacy) for CERHR Evaluation Process: This study has utility in showing a NOAEL of 100 mg/kg bw/day and a LOAEL of 200 mg/kg bw/day under the exposure conditions of the experiment.

Butcher et al. (1973) examined the effect of prenatal hydroxyurea exposure on neurobehavioral effects in rats. Wistar rats were i.p. injected with hydroxyurea at 0, 375, or 500 mg/kg bw on GD 12 (GD 0 = day of vaginal sperm). Controls were not injected. **[Purity of hydroxyurea and number of dams/group were not reported.]** Five hours after hydroxyurea administration, dams were anesthetized, implantation sites were counted, and a few embryos were removed and fixed in Bouin solution for examination of necrosis. One embryo/litter was used to determine the hydroxyurea level. Females were allowed to litter normally, and all litters were examined at birth. To distinguish between prenatal and postnatal effects, half of the treated and control litters were cross-fostered on the day after birth. Offspring were weaned at 25 days of age. Between 30–40 days of age, exploratory behavior was examined in all offspring (55 in the control group, 48 in the 375 mg/kg bw/day group, and 19 in the 500 mg/kg bw/day group). Ten days later, swimming ability was assessed and the animals were tested in a water maze.

Methods of statistical analyses included *t*-test, ANOVA, and Scheffé test.

Appreciable levels of hydroxyurea were detected in embryos from all litters but one, which was dropped from analysis. **[Actual concentrations of detected hydroxyurea were not reported.]** Examination of neural tubes in embryos hr after treatment showed slight cytotoxicity at 375 mg/kg bw, moderate cytotoxicity at 500 mg/kg bw, and reduced mitotic figures in the ependymal layer of the neural tube at both doses. Litter sizes at birth were equivalent across the control and dose groups. There was no evidence of external malformations at birth. There was no significant effect of hydroxyurea on open-field behavior. Two abnormalities that were not observed at birth were noticed during the open-field testing. In the hydroxyurea groups, there was an increase in the number of rats (percentages affected in control and each treatment group) that lacked normal neuromuscular control of hindlimbs (0, 21, and 23%) or had kinked tails (0, 6, and 47%). **[The low-dose kinked tail response was not different from control by Fisher exact test carried out by CERHR. Modeling the proportion of pups with splaying, BMD_{10} = 219 mg/kg bw and $BMDL_{10}$ = 168 mg/kg bw. For kinked tail, BMD_{10} = 394 mg/kg bw and BMD_{10} = 355 mg/kg bw.]** Rats were weighed before maze testing, and body weights were reported to be significantly reduced at the high dose in female rats **[by 14%]** and in both sexes combined. **[The mean body weights were not shown for sexes combined, and the numbers of males and females were not given, precluding BMD modeling.]** No effects were observed in swimming speed trials, but rats in both dose groups made significantly more errors in the water maze test **[19 and 61% more than controls]**. Cross-fostering had no significant effect on any endpoint examined. The study authors concluded that prenatal hydroxyurea exposure at doses that cause only minor malformations altered postnatal functional capacity of rats.

Strengths/Weaknesses: The use of physical postnatal endpoints (e.g., physical maturation endpoints, body weight, etc.) with multiple, sensitive behavioral tests during the postnatal period after exposure to two reasonable dose levels is a strength in this study. Correlation of these endpoints with outcome is useful. Other strengths are the use of multiple dose levels, the use of histology in embryos 5 hr after exposure, and cross-fostering to separate prenatal and postnatal effects. The dosing period of a single day of gestation is a weakness, giving rise to the question of whether the most susceptible period for affecting the central nervous system development and thus behavior was selected. The authors claimed to have information on internal doses, although no data were presented.

Utility (Adequacy) of CERHR Evaluation Process: This study is useful in identifying new endpoints of hydroxyurea toxicity, but lower dose effects were not identified. The utility of the study is limited by the single day of dosing.

Adlard and Dobbing (1975), support not indicated, examined the effect of prenatal hydroxyurea exposure on neurobehavioral function of rats. On GD 14 (GD 0 = day of vaginal sperm), black and white hooded rats were i.p. injected with saline vehicle (n = 15) or hydroxyurea **[purity not reported]** at 1000 or 2000 mg/kg bw (n = 7/group). Litters were culled to four pups/sex on the day of birth, and pups from the control and treated groups were fostered to a saline-treated dam that had given birth on the same day. Body weight, brain weight, and brain DNA content were measured in culled pups (n = 12–20/pups/group from 15 control litters and from 7 treated litters/group). Unless otherwise specified, the remaining analyses were conducted in pups obtained from 15 control litters, seven litters from the low-dose group, and four litters from the high-dose group; results in pups from hydroxyurea groups were pooled. Half the pups from each litter were killed at 25 days of age, and whole brain and cerebellum were weighed in 25 control pups and a pooled hydroxyurea group of 19 pups. The remaining pups, up to 2/sex/litter, were weaned. Brain and body weights were also measured at 18 weeks of age in 12 rats/sex from the control group and a pooled hydroxyurea group of 7–10 offspring/sex. Offspring were tested in a Hebb-Williams maze at 13–15 weeks of age (n = 12 controls/sex and 7–10/pooled hydroxyurea-treated rats) and in a water T-maze at 20–23 weeks of age (n = 5–6/sex/group from 6 litters/group). Data were analyzed by Student's *t*-test.

Table 46
Effects at Birth in Rat Pups Exposed to Hydroxyurea During Gestation

	Hydroxyurea dose (mg/kg bw)	
Endpoint	1000	2000
Body weight[a]	↓27%	↓32%
Brain weight[a]	↓28%	↓30%
Brain DNA content	↓34%	↓33%
Brain:body weight ratio	↔	↔
Neonatal mortality at 48 hr	↔	↑[b]

From Adlard and Dobbing (1975).
[a]Benchmark doses were not calculated, because the Expert Panel did not believe that a dose–response relationship had been adequately demonstrated.
[b]Complete death in 3 of 7 litters.
↓,↑, Statistically significant decrease or increase, respectively, compared to controls; ↔, no statistically significant effect compared to controls.

No adverse effects on maternal weight gain, signs of toxicity in dams, or reductions in numbers of liveborn pups were observed in the hydroxyurea-treated groups. **[Data were not shown.]** Kinked tail was the only malformation observed in the hydroxyurea-treated groups **[numbers of affected pups and litters not reported]**. Offspring exposed to hydroxyurea also had reduced coat pigmentation. As summarized in greater detail in Table 46, both of the hydroxyurea doses decreased body and brain weight and brain DNA content in 1-day-old pups, and the high dose decreased 48-hr survival. Because there were no differences in growth at PND 25 between the two hydroxyurea groups, the two treatment groups were pooled in further analyses of growth and neurobehavior. Results of the pooled hydroxyurea groups are summarized in Table 47. As noted in Table 47, hydroxyurea-induced decreases in body and brain weight continued through 25 days and 18 weeks of age. Rats from the hydroxyurea group made more errors in the Hebb-Williams maze test, but running time was not affected. Significantly fewer errors were reported to have been committed by rats with brain weights

>1250 mg. On the first 4 days of the T-maze test, when the escape route was kept constant, there was no effect of hydroxyurea treatment. When the escape route was reversed on Testing Day 5, rats from the hydroxyurea groups made fewer errors than controls, but during the next four trials conducted on Day 5, more errors were made by rats in the hydroxyurea group. The study authors concluded that prenatal exposure to hydroxyurea resulted in permanent inhibition of brain growth and impaired learning ability in rats.

Strengths/Weaknesses: Strengths are the corroboration of the kinked tail effect seen with other strains and the use of neurobehavioral endpoints, including brain weight. The study design (sequential testing of littermates) improved the ability to detect changes, especially litter-specific changes. The authors used a good testing paradigm, specific motor activity tests during the dark phase, when the animals are the most active. Overall, the authors used very good techniques to examine the behavioral endpoints. However, the dosing period was only on a single day of gestation, leaving the question of whether the most susceptible period to affect the central nervous system development and thus behavior was selected. The authors claimed to have information on internal doses, although no data were presented. The authors pooled the data from treated animals (receiving 1000 or 2000 mg/kg), which would have introduced a fair amount of variability into the data. The doses were much higher than doses shown previously to be teratogenic at earlier exposure times, and no NOAEL was identified.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Table 47
Growth and Neurobehavioral Effects in Rats Prenatally Exposed to Hydroxyurea

Endpoint	Pooled hydroxyurea groups compared to control
Body weight, PND 25	↓20%
Brain weight, PND 25	↓31%
Cerebellum weight, PND 25	↓ 19%
Brain:body weight ratio, PND 25	↓**[17%]**
Cerebellum:whole brain ratio, PND 25	↑**[19%]**
Male body weight, 18 weeks old	↓32%
Male brain weight, 18 weeks old	↓33%
Male brain:body weight ratio, 18 weeks old	↔
Female body weight, 18 weeks old	↓15%
Female brain weight, 18 weeks old	↓31%
Female brain:body weight ratio, 18 weeks old	↓18%
Errors, Hebb-Williams test	↑28%
Errorless runs, first day of T-maze reversal	↓
Errors, first trial on first day of T-maze reversal	↓ 3-fold
Errors, 10 trials on first day of T-maze reversal	↑(53 vs. 15% in controls)

From Adlard and Dobbing (1975).
↑,↓, Statistically significant increase or decrease, respectively; ↔, no statistically significant effect. PND, postnatal day.

Asano et al. (1983), support not indicated, examined behavior in rats exposed to hydroxyurea during prenatal development. Two sets of studies were conducted; in both, Wistar rats were i.p. injected with hydroxyurea **[purity not given]** or the saline vehicle. GD 0 was defined as the day of vaginal sperm. Dams were allowed to deliver and raise their offspring. On the Day 4 after delivery, litters were culled to eight pups, with equal numbers of males and females when possible. The litter was considered the experimental unit in statistical analyses. Data were analyzed by Kruskal-Wallis test, ANOVA, and Scheffé test.

In the first experiment, 10–12 dams/group were i.p. dosed with hydroxyurea at 0, 25, 50, or 100 mg/kg bw/day on GD 9–12. Dose selection was based on results of preliminary testing that showed effects on fetal weight and malformations at 200 mg/kg bw/day hydroxyurea given on GD 9–12. A morphologic examination of brain was conducted in culled offspring. Reflex development was examined in surviving offspring. Open-field and rotorod performance were examined in one offspring/sex/litter. Avoidance behavior was tested in one offspring/sex/litter in the control and the two highest dose groups. Hydroxyurea exposure had no effect on delivery index, stillbirth, or pup body weight and viability through 21 days of age. Dilated lateral ventricles occurred in one to four culled pups/group. Effects that attained statistical significance or appeared to be treatment-related according to the study authors' descriptions are summarized in Table 48. Although no malformations

Table 48
Developmental Toxicity in Offspring of Rats Exposed to Hydroxyurea on GD 9–12

	Hydroxyurea dose (mg/kg bw/day)				
Endpoint	25	50	100	BMD_{10}[a]	$BMDL_{10}$
External malformation incidence, PND 4[b]	↔ (4.4%)	↔ (7.7%)	↑ to 18%	74	60
External malformation incidence, PND 21[b]	↔ (1.3%)	↔ (1.2%)	↑ to 16.5%	86	75
Female righting reflex, PND 2	↓	↔	↔	—	—
Male free-fall reflex, PND 15–25	↔	↔	↓	—	—
Female rearing, 4 weeks old	↔	↔	↑73%	—	—

From Asano et al. (1983).
[a]For a discussion of the use of benchmark dose in this report, see footnote to Table 33. A probit model was used.
[b]No malformations were observed in controls.
↑,↓, Statistically significant increase or decrease, respectively, compared to controls; ↔, no difference compared to controls. GD, gestational day; PND, postnatal day.

Table 49
Developmental Toxicity in Offspring of Rats Exposed to Hydroxyurea on GD 9–12

	Hydroxyurea dose (mg/kg bw/day)			
Endpoint	100	200	BMD_{10}[a]	$BMDL_{10}$
Stillbirth incidence	↔	↑ to 25.7% (control 1.8%)	135	119
Male pup birth weight	↔	↓8%	271[b]	146[b]
External malformations[c]				
At birth	↔ (0%)	↑ to 20.2%	188	158
PND 4	↔ (5.3 %)	↑ to 69.2%	116	92
PND 14	↔ (5.9%)	↑ to 87.5%	110	79
PND 21	↔ (0%)	↑ to 62.5%	174	110
PND 56	↔ (7.5%)	↑ to 68.8%	109	84
Viability index, PND 56	↔	↓ to 66.7% (control 100%)	284	138
Male free-fall reflex, PND 16–21	↔ (87.5%)	↓ to 33% (control 97%)	101	72

From Asano et al. (1983).
[a]For a discussion of the use of benchmark dose in this report, see footnote to Table 33. A probit model was used. Viability index was converted to viable pups at postnatal day (PND) 56/viable pups at PND 21 for the BMD analysis. Data were presented on a per fetus basis, and benchmark dose analysis may be less meaningful without consideration of litter of origin.
[b]For this endpoint, the BMD_{1SD} was 231 and the $BMDL_{1SD}$ was 120 mg/kg bw/day.
[c]No malformations were observed in controls.
↑,↓, Statistically significant increase or decrease, respectively, compared to controls; ↔, no statistically significant difference compared to controls. GD, gestational day; PND, postnatal day.

were observed at birth, external malformations were observed during the postnatal period in the 100 mg/kg bw/day group. Types of malformation included anophthalmia at 4 days of age and anophthalmia and enlarged cranial vault at 21 days of age. Delays were observed in righting reflex in low-dose females at 2 days of age and free-fall reflex in high-dose males at 15–25 days of age. Rearing frequency in high-dose females in open-field testing was increased at 4 weeks but not at 12 weeks of age. No significant effects were reported for performance on rotorod at 4 weeks of age or avoidance testing at 6–7 weeks of age.

In the second study, 8–10 rat dams/group were i.p. dosed with 0, 100, or 200 mg/kg bw/day hydroxyurea on GD 9–12. Offspring were monitored for development of free-fall reflex. Macroscopic examination of the brain was conducted at 4 days of age in all culled offspring, at 12 and 21 days of age in one offspring/sex/litter, and at 56 days of age in all remaining offspring. Effects of hydroxyurea treatment are summarized in Table 49. All treatment-related effects were observed at the high dose and included decreased birth weight of male pups and increased stillbirth and external malformations. Malformations observed in both dose groups included eye defects (pannus, corneal opacity, anterior synechia, microphthalmia, and anophthalmia) and dilated brain ventricles, but the apparent small increase at the low dose did show statistical significance. Attainment of free-fall reflex was delayed in high-dose males. According to the study authors, delays in attaining free-fall reflex were of greater magnitude in rats with ventricular dilatation.

The study authors concluded that although the malformation rate in the 100 mg/kg bw/day group was higher in the first than the second study, the types of malformations were consistent in the two studies. No statistically significant differences were observed for free-fall reflex between the first and second studies. The study authors concluded that hydroxyurea induced morphologic and behavioral abnormalities and that there was very little difference in the dose required to produce either abnormality.

Strengths/Weaknesses: The authors investigated a relevant portion of the dose-response curve and susceptible stage of development to evaluate multiple morphologic and behavioral outcomes. They correlated functional outcome with morphologic endpoints, which is important in trying to understand the dose-response relationships of these endpoints. Comparison of this study to morphologic data from other studies, e.g., Price et al. ([1985a,b) allows confirmation of effects due to dosing with hydroxyurea from GD 7 through GD 20 (effects at 200 mg/kg bw/day). The lack of exposure in the current study (the various dose levels were not administered over the entire period of gestation) does not allow determination of whether the sensitive period for behavioral outcomes was covered. Another weakness is the assessment of only external defects.

Utility (Adequacy) for CERHR Evaluation Process: This study has utility in this evaluation in providing information on the lower portion of the dose-response curve, giving a LOAEL of 100 mg/kg bw/day, a NOAEL of 50 mg/kg bw/day, a BMD_{10} of 74 mg/kg bw/day, and a $BMDL_{10}$ of 60 mg/kg bw/day.

Asano et al. (1985), support not indicated, examined developmental neurotoxicity effects in rats exposed prenatally to hydroxyurea. On GD 9–12 (GD 0 = day of vaginal sperm), 14–16 Sprague-Dawley rats/group were i.p. injected with 50 or 100 mg/kg bw/day hydroxyurea **[purity not given]** in saline. The control group was not treated. Dams were allowed to deliver and nurse their litters. At birth, offspring were culled to eight offspring, six males and two females when possible. Male pups were examined for external malformations, weighed during the lactation period, and examined for developmental milestones, reflex development, open-field activity, and rotorod performance. Numbers of pups

examined were 83–119/group for body weight and malformations and 14–16/group (1/litter) in open-field testing. The litter was considered the experimental unit in statistical analyses. Data were analyzed by Kruskal-Wallis test, ANOVA, Scheffé test, or χ^2.

Hydroxyurea exposure had no significant effect on delivery index, stillborn pups, or postnatal body weight gain. Microphthalmia observed in 2/119 high-dose pups on PND 21 was the only malformation reported. Hydroxyurea had no significant effect on eyelid opening, traction response, righting or free-fall reflex, or rotorod performance. **[Data were not shown.]** Some significant ($P < 0.05$) effects were observed for ambulation in the open-field testing that was conducted for 2 days at 3, 4, 5, 7, and 11 days of age. Decreased ambulation episodes were observed in the mid-dose group on the first day of testing at 4 weeks of age **[29% fewer than controls]** and on the second day of testing at 3 weeks of age **[48% fewer]**. Ambulation episodes were reduced on the second day of testing in 5-week-old high-dose animals **[21% fewer]**. The study authors concluded that exploratory behavior was suppressed in Sprague-Dawley rats exposed to hydroxyurea during prenatal development.

Strengths/Weaknesses: The investigators used relevant dose levels with adequate group sizes to determine possible outcomes. However, the use of a relatively short dosing period (not throughout the entire period of gestation) and the lack of treated controls are weaknesses of this study. In addition, only male pups were examined for behavioral outcomes.

Utility (Adequacy) for CERHR Evaluation Process: The study has utility in that it confirms the presence of microphthalmia (at a very low rate) after exposure to 100 mg/kg hydroxyurea.

Brunner et al. (1978), supported by FDA, examined the effects of prenatal hydroxyurea exposure on behavior and physical abnormalities in rats. Sprague-Dawley rats were injected with a single dose of 150 mg/kg bw hydroxyurea in distilled water on GD 6, 9, 12, 15, or 18 (GD 0 = day of vaginal plug). **[Purity of hydroxyurea and the specific route of injection were not reported.]** Control rats were not injected. Based on unpublished data, the hydroxyurea dose was expected to produce malformations when administered on GD 9. Large litters were culled to 10 or 11 pups at birth, and litters with fewer than eight pups were not evaluated. At birth, pups were weighed and examined for external malformations. From 2–21 days of age, the pups were examined for surface righting, mid-air righting, cliff avoidance, swimming ability, pivoting, and startle response. A total of 60 litters were evaluated for behavioral and weight effects. **[Based on a footnote in** Table 1 **of the study, it appears that 10 litters/group were examined.]** Rats were weighed and killed at 21 days of age. Eyes and brain were weighed. In analyses of data, the litter was considered the statistical unit. **[Statistical analyses were not discussed, but according to the Results section it appears that ANOVA was used.]**

Exposure to hydroxyurea at any time point did not significantly affect body weight at birth or at 21 days of age. There were no statistically significant effects of hydroxyurea on age of attainment of surface righting, mid-air righting, cliff avoidance, and startle response or frequency or time of swimming behaviors or pivoting responses. Increased incidences of hydrocephalus (in 1 or 2 pups/litter from 5 litters) and microphthalmia **[incidence not reported]** were observed in offspring of rats exposed to hydroxyurea on GD 9. Eyes weighed significantly less **[16% in males and 6% in females]** on PND 21 in rats exposed on GD 9, and the study authors stated that this effect confirmed microphthalmia. Exposure to hydroxyurea had no significant effect on cerebellum, cerebrum, or brain stem weight. The study authors concluded that behavioral endpoints are not necessarily more sensitive than morphometric measurements and that behavioral tests need to include assessments of brain pathology to bridge structural and functional approaches.

Strengths/Weaknesses: The study confirms the eye malformations that were previously observed, and 150 mg/kg bw/day was a NOAEL for behavioral endpoints. The authors weighed the tissue from the eye, allowing a quantitative dose response for microphthalmia or anophthalmia. Although the evaluation of multiple days of exposure is a strength, the dosing period was for only a single day of gestation and only a single dose level was used. Although several different days of gestation were tested, it is still not clear whether the most sensitive day of development or the lowest effective dose level were used. The lack of incidence data is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study has utility only in confirming what has been observed in previous studies.

Vorhees et al. (1979), supported by FDA, examined the effectiveness of hydroxyurea as a positive control in neurobehavioral testing. Two other compounds examined will not be discussed here. Sprague-Dawley rats (n = ~21/group) were i.p. injected with 550 mg/kg bw hydroxyurea **[purity not given]** on GD 12. A negative control group of ~20 dams was not treated. Gestation length was assessed, and pups were counted, sexed, and examined for viability at birth. Litters with fewer than eight offspring were discarded. Other litters were culled to no more than 12 pups, with equal numbers of males and females when possible. At 1 day of age, two pups/sex/litter were designated for preweaning testing, and at weaning, two pups/sex/litter were selected for postweaning testing. **[Numbers of pups tested were not indicated, but the numbers of litters represented were reported in most cases.]** During the lactation period, pups were weighed and monitored for attainment of developmental milestones, reflexes, and swimming, locomotion, and visual-placement ability. In the postweaning period, rats were evaluated for performance on activity-wheel, rotorod, avoidance, open-field, and maze tests. Brain histopathology was examined in six males/group at weaning and in an unspecified number of males at 90 days of age. Brain and eye weights were measured in 90-day-old males. Statistical analyses included ANOVA, Fisher test, Newman-Keuls test, and Kramer test.

No significant differences were reported for maternal weight during the gestation or lactation periods. **[Data were not shown.]** In most cases, pups from 11–21 litters/group were available for the various evaluations conducted. During the lactation period, the mortality rate of offspring was reported at 12.5% in the hydroxyurea group and 5.8% in the negative control group **[statistical significance not indicated by study authors]**. Body weights in the hydroxyurea group compared to the

control group were lower **[by 11–19%]** in male and female offspring during the lactation period and in females **[by 13%]** at 45 days of age. In the postweaning period, feed intake was lower **[by 13%]** in male and **[by 21%]** in female offspring from the hydroxyurea group. No significant effects were reported for developmental landmarks, but a 0.7-day delay in eye opening was said to approach statistical significance in the hydroxyurea group. Auditory startle reflex was delayed by 1 day in the hydroxyurea group. At 6, 8, or 10 days of age, offspring in the hydroxyurea group performed worse than negative controls on swimming test endpoints such as direction, angle, and limb usage. In the hydroxyurea group, decreased rearing frequency during open-field testing was the only effect reported in postweaning neurobehavioral tests. Neuron numbers were decreased **[by 11%]** in the cerebellum but not in the olfactory bulb or hippocampus of 21-day-old males from the hydroxyurea group. Effects observed in 90-day-old males exposed to hydroxyurea included an 11% decrease in cerebellum weight, a 13% decrease in brain stem weight, an 18% decrease in cerebrum weight, and a 6% reduction in eye weight. Hydroxyurea did not affect dendritic spine counts in Golgi-Cox cortical sections prepared from 90-day-old rats. All other endpoints examined were unaffected by hydroxyurea treatment. The study authors concluded that the pattern of effects was less severe than expected after hydroxyurea exposure, thus indicating that hydroxyurea is an adequate but not optimal positive control for neurobehavioral testing.

Strengths/Weaknesses: The investigators used a battery of behavioral tests and measured brain weights in an attempt to explore subtle effects of prenatal hydroxyurea exposure, and the assessment over a long period is a strength. However, the use of a single high dose level of hydroxyurea on a single day of gestation does not provide information for a dose-response curve.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited utility.

Fritz and Hess (1980), from Ciba-Geigy Limited, examined the effects of hydroxyurea exposure on postnatal growth and behavior of rats. On GD 14 (GD 0 = day of vaginal sperm or plug), at least 7–10 Sprague-Dawley rats/group were i.p. injected with saline vehicle or 2000 mg/kg bw hydroxyurea **[indicated "purum"]**. Pregnancy duration was assessed, and pups were sexed and evaluated for viability at birth. During the postnatal period, pups were weighed and examined for developmental and behavioral endpoints, including as developmental landmarks, attainment of reflexes, activity, and hearing. Pups were weaned on PND 28 (day of birth not defined). On PND 60, 10–15 offspring/sex from seven to eight litters/group were selected randomly for autopsy and measurement of brain weight. Statistical analyses included χ^2 with Yates correction or Student's *t*-test.

Pregnancy duration was normal (21–22 days), and dams displayed no signs of toxicity. One control dam and two treated dams aborted. One litter from a treated dam died on PND 1. Hydroxyurea treatment did not affect litter size or sex ratio. Postnatal mortality was increased in the hydroxyurea group (27% compared to 1.2% in controls). Weight gain was reduced in the hydroxyurea group **[by ~15% compared to controls]**. Oligodactyly of the rear paw was observed in one pup of the treated group, and that pup died on PND 3. Three pups from two litters in the treated group had not achieved righting reflex by PND 14. Fewer pups in the treated group than in the control group were able to climb a wire mesh wall on PND 32 (14 vs. 37%, $P \leq 0.01$). Activity index was reduced in the treated group (1.4 vs. 2.0 in controls). **[The effect on activity index was not reported to be statistically significant in Table 2 of the study, but the study authors indicate the effect to be significant in the abstract.] Exposure to hydroxyurea did not affect nesting behavior, eye opening, pinna detachment, pupillary constriction, hearing ability, or brain weight. At 64 days of age, 11 males and 22 females from seven litters/group were randomly selected for mating of one male to two females from another litter. Rats were mated over a 10-day period that included at least one estrous cycle. Females were killed and necropsied on Day 14 post-coitum. Implantation sites were assessed for viability and counted. No significant differences were reported for fertility rates or numbers or viability of implantation sites.**

The study authors concluded that prenatal hydroxyurea exposure induced postnatal mortality, possibly impaired postnatal growth, and affected locomotor activity but did not impair overall sensorial development of rats. They also concluded that that prenatal exposure to hydroxyurea did not appear to affect germinal cells of rats.

Strengths/Weaknesses: The authors used several atypical tests for behavioral endpoints and had adequate group sizes. The corroboration of hindlimb effects shown in a previous study is a strength. However, the use of a very high dose level of hydroxyurea on a single day of gestation resulted in significant postnatal mortality, allowing for only a limited number of pups available for measures of postnatal function and growth.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Vorhees et al. (1981), support not indicated, used hydroxyurea as a positive control in a study to examine neurobehavioral effects of butylated hydroxytoluene. The study of butylated hydroxytoluene will not be discussed here. On GD 12, Sprague-Dawley rats (n = 17) were i.p. injected with 550 mg/kg bw hydroxyurea **[purity not given]**, and at least 19 negative control dams were untreated. Dams were weighed during the study and gestation length was recorded. Dams were allowed to litter, and litters with fewer than eight pups were discarded. Litters were culled to no more than 12 pups, with equal numbers of each sex when possible. On the day after birth (PND 1), litters were assessed for size, sex distribution, weight, viability, and malformations. Developmental milestones, acquisition of reflexes, locomotion, open-field behavior, and swimming ability were examined before weaning in ~2 pups/sex/litter, with the exception of swimming ability for which ~1 pup/sex/litter was examined. Post-weaning observations in ~1 pup/sex/litter included open-field behavior, running-wheel activity, rotorod balancing, and active and passive avoidance tests. Eye and brain weights were measured on PND 90 in one male offspring/litter. The litter was considered the statistical unit in analyses of preweaning data, and the individual animal was considered the statistical unit for the evaluation of postweaning data. Statistical analyses

included Fisher test and ANOVA followed by Duncan comparisons when statistical significance was obtained.

No significant effects on body weight during the gestation or lactation periods were reported for dams exposed to hydroxyurea. Gestation length and sex ratio were also reported to be unaffected by hydroxyurea exposure **[data not shown by study authors]**. Only seven litters from the hydroxyurea group were available for postnatal evaluation, apparently because of a high resorption rate. During the preweaning period, body weights of offspring were lower in males and females of the hydroxyurea group on PND 7 and females on PND 21 **[~15–19% lower than controls]**. Hydroxyurea exposure did not affect body weight gain of offspring in the postweaning period. On PND 1–30, pup mortality was described as increased in the hydroxyurea group compared to the control group (10 vs. 3%), but the effect did not achieve statistical significance. In evaluations conducted in the preweaning period, offspring in the hydroxyurea group experienced a ~$\frac{1}{2}$-day delay in eye opening, a ~2-day delay in development of forward locomotion, and delayed onset of four-legged swimming in males. Exposure to hydroxyurea did not significantly affect any endpoint evaluated in the postweaning period, including performance in open-field, activity-wheel, rotorod, or avoidance testing. On PND 90, there were significant reductions (% change compared to controls) in weights of the cerebrum (15.5%), total brain (14%), and eye (5%) in males from the hydroxyurea group. The study authors concluded that although prenatal hydroxyurea exposure affected some endpoints in rat offspring, it was not an ideal positive control.

Strengths/Weaknesses: The investigators used several atypical tests for behavior to detect developmental outcomes. However, the use of a single high dose level of hydroxyurea on GD 12 (as a positive control) resulted in significant postnatal mortality, leading to a very small group size for postnatal evaluation and limiting the study's utility for this evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited utility.

Vorhees et al. (1983a), supported in part by FDA, used hydroxyurea as a positive control in a study to evaluate postnatal developmental toxicity induced by FD&C Red Dye #3 in rats. The experiment for FD&C Red Dye #3 will not be discussed here. On PND 2–10 (day of birth = PND 0), Sprague-Dawley rats were s.c. injected with 50 mg/kg bw/day hydroxyurea **[purity not given]**. Negative controls were not treated. The rats were evaluated for postnatal mortality. Neurobehavioral effects were evaluated using the Cincinnati psychoteratogenicity test to assess reflex acquisition, swimming ability, open-field behavior, performance on rotorod and running wheel, and active and passive avoidance. Day of vaginal opening was also monitored. **[The numbers of animals treated and evaluated were not specified, but it appears that treated offspring were obtained from 10 dams and control offspring were obtained from 18 dams. Table 4 of the study indicated that 19 litters were represented in the hydroxyurea group. It is possible that this number actually represented the number of pups tested. Although the method section states that rats were treated only during the postnatal period, some endpoints of prenatal or dam toxicity were presented for the hydroxyurea group. It is assumed the authors were referring to dams and litters pre-selected for hydroxyurea exposure in the postnatal period. Protocol details were limited, and it was not clear if rats exposed to hydroxyurea were examined for all endpoints discussed.] Data were analyzed by Fisher test or ANOVA followed by Duncan a posteriori multiple range comparison test when statistical significance was obtained.**

Offspring mortality on PND 1–21 was increased in the hydroxyurea compared to the negative control group (8.6 vs. 3.2%). No effect was reported for postnatal weight gain after hydroxyurea exposure. **[Data were not shown.]** Postweaning ambulation was said to be increased in the hydroxyurea group. Vaginal opening was delayed by 4.9 days in females exposed to hydroxyurea. No other significant neurobehavioral effects were reported for the hydroxyurea group. **[With the exception of data for swimming tests, no data were shown for neurobehavioral endpoints.]** The study authors concluded that postnatal exposure to hydroxyurea resulted in similar but milder effects as compared to results form previous prenatal exposure testing conducted in their laboratory.

Strengths/Weaknesses: A battery of atypical behavioral tests was used with a large number of test subjects per group to determine possible effects of postnatal hydroxyurea exposure. However, the use of s.c. injections to administer the hydroxyurea raises questions regarding possible differences in pharmacokinetics, especially during the early postnatal stage of life. The selection of the early postnatal period for hydroxyurea exposure is important, because many developmental events correlate to the third trimester in humans. Significant pre-weaning mortality limited the number of test subjects available for the developmental endpoints. The effects observed were mild and less than if the exposure had been prenatal.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Vorhees et al. (1983b), supported in part by FDA, used hydroxyurea as a positive control in studies examining neurobehavioral effects in rats exposed to FD&C Red No. 40. The effects of FD&C Red No. 40 will not be discussed here. Sprague-Dawley rats obtained from 10 litters were s.c. injected on PND 2–10 with 50 mg/kg bw/day hydroxyurea **[purity not given]**. Negative controls obtained from 15 litters were not treated. The rats were evaluated for postnatal mortality, body weight gain, developmental milestones, and vaginal opening. Neurobehavioral effects were evaluated using the Cincinnati psychoteratogenicity test, which assessed reflex acquisition, swimming ability, open-field behavior, performance on rotorod and running wheel, and active and passive avoidance. Brain weights were measured on PND 90. The study authors indicated that two males and female/litter were designated for testing in the preweaning period and two males and females/litter were designated for testing in the postweaning period. **[**Table 4 **in the study indicates that nine males (<1/litter) were tested in running-wheel activity.]** Statistical analyses included Fisher test or ANOVA followed by Duncan a posteriori multiple-range comparison when statistical significance was achieved.

Pre- and postweaning body weights were reduced in both males and females of the hydroxyurea group. The "weight difference" was reported at 16.7% in males on

PND 42 and 14.0% in males on PND 90. The study authors noted that dam body weights were also reduced during the lactation period, but because the dams received no hydroxyurea, it was noted that the effect was not treatment-related and of was unknown cause. Swimming and paddling development were delayed on PND 6 in the hydroxyurea group. Vaginal opening was delayed by 8 days in the hydroxyurea group. In three of five blocks of running-wheel activity testing conducted on PND 30–50, activity was reduced **[by 36–46%]** in males of the hydroxyurea group. Cerebellar weight was reduced by 7.9% in males on PND 90, and a similar effect was reported in females. None of the other endpoints were affected by hydroxyurea exposure. **[Data were not shown for endpoints unaffected in the hydroxyurea group.]** The authors did not express conclusions about the effectiveness of hydroxyurea as a positive control.

Strengths/Weaknesses: This study used a battery of apical behavioral tests to investigate hydroxyurea effects on behavioral teratogenicity. The study used an appropriate number of animals per group, and used an appropriate study design. The use of a s.c. injection of hydroxyurea during the postnatal period (PND 2–10) would correlate with the third trimester of human gestation. The information provided evidence of subtle effects on behavioral endpoints later as the animals matured. The usefulness of this study is enhanced by the dosing period selected; however, the differences between the kinetics after s.c. injection versus what a third trimester human fetus would receive needs to be resolved.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Brock et al. (1980), supported by the National Science Foundation and the National Cancer Institute, examined the effects of hydroxyurea on testicular histones of rats. Twenty-day-old rats were i.p. injected three times with 300 mg/kg bw hydroxyurea **[purity not given]** at 1-hr intervals. Concurrent with the second hydroxyurea injection, rats were also given an intratesticular injection of ^{3}H-lysine and ^{14}C-thymide. **[A control group was included, but treatment of that group was not described. The number of rats treated was not reported, but it was stated that seven experiments were conducted.]** After a 1-hr labeling period, proteins were isolated from testes. Histones were separated by electrophoresis, testicular DNA was measured for ^{14}C activity, and testicular proteins were measured for ^{3}H activity. Data were analyzed by Student's *t*-test. In treated rats, ^{14}C-thymidine was incorporated into DNA at 1% of control levels. Compared to controls, hydroxyurea-treated rats produced less H3-, H2B-, H2A-, and H4-type histone (22–70.4% of control levels). There was no significant effect on synthesis of TH1-, H1-, and TH2B-type histones. The study authors concluded that hydroxyurea has varying effects on production of different types of histones and that synthesis of some histones is not coupled to DNA synthesis.

Strengths/Weaknesses: The study design used an extremely high dose level of hydroxyurea in sexually immature rats. The endpoints that were measured are of questionable significance for the purpose of this evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation process.

Gupta and Yaffe (1982), support not indicated, examined the effect of hydroxyurea exposure on the development of the female reproductive tract. The focus of the study was the determination of the effects of DNA or protein synthesis inhibitors on phenobarbital-induced developmental toxicity. Chemical exposures not involving hydroxyurea will not be discussed here. On GD 17–20 (day of vaginal sperm not indicated), five Sprague-Dawley rats/group were s.c. injected with saline, 160 mg/kg bw/day hydroxyurea **[purity not given]**, or 160 mg/kg bw/day hydroxyurea+40 mg/kg bw/day phenobarbital. When hydroxyurea and phenobarbital were co-administered, hydroxyurea was injected in divided doses, at the time of phenobarbital treatment and then 6 hr later. At an unspecified time after birth, litters were culled to 8–10 pups. Onset of puberty was examined in 18–22 offspring/group. Estrous cyclicity was monitored for 12 consecutive days in 5–11 offspring/group, beginning at 60 days of age. At 80–90 days of age, 6–13 female offspring/group were evaluated for fertility by mating with untreated males for 5–15 days, until vaginal sperm were observed. Fertility was determined by examining implantation sites **[at an unspecified time period of pregnancy]**. Rats that were not evaluated for fertility (n = 4–7/group) were killed during estrous at 3–4 months of age for determination of plasma estrogen levels using a radioimmunoassay (RIA) method. Vaginal opening and plasma 17β-estradiol level data were analyzed by Student's *t*-test. Estrous cycles and fertility data were analyzed by χ^2 test with Yates correction.

No gross malformations or effects on body weight were observed after exposure to hydroxyurea alone. **[Data were not shown.]** In rats treated with hydroxyurea alone, there were no effects on onset of puberty, estrous cycles, fertility, or plasma 17β-estradiol levels. Co-administration of hydroxyurea inhibited effects observed with administration of phenobarbital alone, including delayed onset of puberty, estrous cycle irregularities, reduced fertility, and increased plasma 17β-estradiol levels. The study authors concluded that prenatal hydroxyurea exposure had no effect on reproductive function of female rat offspring.

Strengths/Weaknesses: The study used a dosing regimen during a fetal period (GD 17–20) that is rarely represented in other studies of hydroxyurea. Dose selection and endpoints measured were appropriate. Although the dosing period did not include the embryonic period, it did use an important period for sexual differentiation in the rat. This study provides limited information on endpoints of postnatal sexual maturity. Use of a single dose level of hydroxyurea at which there was no effect is a weakness.

Utility (Adequacy) of CERHR Evaluation Process: This study is of limited utility.

Amortegui et al. (1976), examined the effects of prenatal hydroxyurea exposure on postnatal hematological values in rats. On GD 15, Sprague-Dawley rats (n = 12) were i.p. injected with saline vehicle and 36 rats were i.p. injected with 1800 mg/kg bw hydroxyurea **[purity not given]**. Rats were allowed to deliver litters and litters were culled to eight pups between 12 and 24 hr after birth. At 1, 5, 10, and 21 days after birth, blood was collected from pups for measurement of hematological endpoints and 2,3-diphosphoglyceraldehyde level. **[No information was provided on the numbers of dams**

treated or pups examined at each time point.] Data were analyzed by Student's *t*-test.

[According to Figures 1 and 2 of the study, numbers of erythrocytes and hemoglobin levels were significantly ($P < 0.05$) higher in hydroxyurea-exposed animals on the first day of life but not at later time periods. In contrast to data reported in figures, the study discussion indicated reduced erythrocyte and hemoglobin levels in rats exposed to hydroxyurea.] No significant effects were reported for leukocyte, hematocrit, mean corpuscular volume, or mean corpuscular hemoglobin concentrations. **[When 2,3-diphosphoglyceraldehyde levels were expressed as µmol/g hemoglobin, levels in the hydroxyurea group were significantly lower compared to the control group only on the first day of life, according to Figure 3 of the study. In contrast, the study discussion indicated that higher levels of 2,3-diphosphoglyceraldehyde/g hemoglobin were observed in the treated group.]** The study authors hypothesized that the effects on erythrocytes and hemoglobin may have resulted from inhibited DNA synthesis and that changes in 2,3-diphosphoglyceraldehyde levels were likely a compensatory response to the decreased hemoglobin levels.

Strengths/Weaknesses: The examination of hematologic endpoints is a strength and represents a relevant endpoint for human for human toxicity after the use of hydroxyurea. The utility of the study is compromised because the text and the tables/graphs within this study do not indicate the same results (opposite, in fact). The authors used a high dose level of hydroxyurea on a single day postnatally and this is of questionable relevance for this evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Prabhakar et al. (1984), supported by the University Grants Commission, New Delhi, examined the effects of hydroxyurea exposure on thymidine kinase activity in neonatal and embryonic Wistar rat brain. In the postnatal exposure study, pups were i.p. injected with hydroxyurea **[purity not given]** at 0 (saline vehicle), 500, 700, or 1000 mg/kg bw/day on PND 4, 5, and 6 (day of birth = PND 1). Pups were killed on PND 7. A total of five to eight experiments/group were conducted; in each experiment, brains from at least two animals were pooled. A time-course study of tyrosine kinase activity was also conducted in pups that were dosed with 0 or 1000 mg/kg bw hydroxyurea and killed on PND 7 at 2, 8, 24, 30, 48, or 72 hr after exposure. Six experiments were conducted, with at least two animals pooled for each experiment. In the prenatal exposure study, dams were i.p. dosed with hydroxyurea at 0 or 1000 mg/kg bw and killed on GD 16 (GD 0 = day of vaginal sperm) at 2, 5, 9, or 20 hr after exposure. A total of 6–12 experiments were conducted in each group, and brains from one or two embryos were used in each experiment. Cerebral hemispheres from pups and embryos were weighed, homogenized, assessed for protein and DNA content, and evaluated for tyrosine kinase activity. **[Statistical analyses were not discussed.]**

No adverse effects (i.e., reductions) in tyrosine kinase activity were observed in brains from rat pups. Specific and total activity of tyrosine kinase was increased in the 700 mg/kg bw/day group, and brain DNA levels were decreased in the 700 and 1000 mg/kg bw/day groups. In the time-course experiment, a decrease in tyrosine kinase activity was only observed at 8 hr after exposure, and increases in activity were observed at 30 and 48 hr after exposure. The study authors concluded that this study showed a lack of consistent hydroxyurea-induced inhibition of tyrosine kinase activity in the cerebrum of 7-day-old rat pups, in contrast to findings in a previous study (Kaplay et al., 1983). In embryonic brain, a **[42%]** decrease in tyrosine-specific activity was observed 5 hr after exposure and **[67–72%]** decreases in tyrosine kinase-specific and total activity were observed at 20 hr after exposure. Decreases in the DNA content of embryonic brain were observed at 9 and 20 hr after exposure. No differences in tyrosine kinase activity were reported after in vitro incubation of hydroxyurea with enzyme preparations from pups or embryos. The study authors concluded that inhibitory action of hydroxyurea on thymidine kinase is dependent on cell proliferation.

Strengths/Weaknesses: This article describes a study of repeated exposures to hydroxyurea with several different dose levels and a single high dose level on a single day of gestation. The examination of thymidine kinase activity after prenatal or postnatal dosing and the corroboration of effects on DNA synthesis are strengths. However, it is not clear if the time examined postnatally was correct for obtaining a maximal effect.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

3.2.2 Mouse. Studies in mice are organized by exposure route.

3.2.2.1 Oral exposure: Roll and Bär (1969), support not indicated, examined the effect of prenatal hydroxyurea exposure on teratogenicity in mice. The study was published in German; CERHR obtained a professional translation. On GD 6–17 (GD 0 = day of vaginal plug), NMRI mice were gavaged with hydroxyurea **[recrystallized numerous times]** at 0 (aqueous vehicle), 5, 10, 15, or 20 mg/animal (200, 400, 600, and 800 mg/kg bw according to the study authors). Some dams were allowed to deliver, and pups were counted and examined for external malformations and viability at birth. Body weights were measured at birth and during the 3-week lactation period. **[In the 5 mg/day dose group, up to three generations of offspring were examined, but no details were provided regarding possible further exposure and mating of offspring in each generation.]** In other groups of dams, fetuses were obtained by cesarean section on GD 18. Implantation sites were examined, and fetuses were assessed for skeletal abnormalities. Data were analyzed by χ^2 and *t*-test. **[The statistical significance of findings was not always explained clearly in the Results section.]**

In the study in which mice were allowed to deliver their offspring, doses of hydroxyurea were 0 (n = 18 dams), 5 (n = 29 dams), and 10 (n = 9 dams) mg/day. An unspecified number of dams were dosed with 15 and 20 mg/animal, but complete resorptions or abortions occurred at those doses. Offspring were also examined in 21 or 22 F_1 and F_2 dams/generation from the 5 mg/day group. Effects of hydroxyurea, summarized in Table 50, included increased offspring deaths at birth and during the lactation period and decreased pup body weight at birth. There were no significant differences in litter sizes at birth or body weights at weaning. **[Findings in the subsequent generations from the 5 mg/animal group**

Appendix II

Table 50
Postnatal Developmental Toxicity in Mice Orally Exposed to Hydroxyurea on GD 6–17

	Hydroxyurea dose, mg/animal (mg/kg bw)	
Endpoint	5 (200)	10 (400)
Stillbirth	↑2. 5-fold	↑3.7-fold
Offspring dying during lactation period	↑2.1-fold	↑2.5-fold
Pup body weight at birth	↓6%	↓ 2%
Malformation	See text for explanation	

From Roll and Bär (1969).
Benchmark doses were not calculated, because the study did not report information needed for modeling.
↑,↓, Statistically significant increase or decrease, respectively, compared to controls. GD, gestational day.

Table 51
Prenatal Developmental Toxicity in Mice Orally Exposed to Hydroxyurea on GD 6–17

	Hydroxyurea dose, mg/animal (mg/kg bw)	
Endpoint	5 (200)	10 (400)
Pups delivered	↓15%	↓94%
Midterm resorption	↑8.3-fold	↑64-fold
Fetal weight	↓27%	No data
Malformation	See Table 52	

From Roll and Bär (1969).
↑,↓, Statistically significant increase or decrease, respectively, compared to controls. GD, gestational day.

were not clear. The study authors stated that a comparison of results from the 5 mg/animal group "…shows that no confirmed differences could be recorded for the 3 successive generations, since, as was already discussed, the test results were homogenous." Results for pup viability and body weight in the subsequent generations appeared to be consistent with those observed in the first generation of animals exposed to the 5 mg/animal dose, suggesting that the values were different from the control values.] Malformations observed at the 5 mg/animal dose included cleft palate in 1.2% of pups and kinked tails in 0.8% of pups. Malformations observed in the 10 mg/animal group were cleft palate in 1.5% and encephalocele in 3% of pups. **[Malformation rates were not provided for the control group.]**

In the study in which fetuses were obtained by cesarean section on GD 18, 16–21 dams/group were treated with hydroxyurea 10 or 20 mg/animal on GD 6–17. There were 150–217 fetuses evaluated in each dose group. Body weight was decreased in fetuses of the 10 mg/animal group; at ≥10 mg/animal, numbers of fetuses were reduced as a result of increased mid-term abortion (Table 51). Incidences of each malformation type observed **[apparently on a fetus and not litter basis]** are summarized in Table 52. The types of malformation observed in the 10 mg/animal group included sternum defects, encephalocele, missing or shortened tail, costal fusion, cervical vertebrae fusion, and malformed thoracic and lumbar vertebra. In the few surviving fetuses available for observation in the 20 mg/animal group, development was morphologically normal but delayed severely.

The same report described treatment of mouse dams with hydroxyurea during specific stages of pregnancy to determine stage sensitivity. Fetuses were examined after delivery by cesarean section on GD 18. Some fetuses were also examined after delivery by natural birth, but very limited details were provided; therefore, this discussion focuses on effects in fetuses obtained on GD 18. The stage-specificity studies included hydroxyurea doses of 15 or 30 mg/animal (600 and 1200 mg/kg bw/day) on GD 6 and 7 (n = 12–18/group); 15 or 30 mg/animal (600 and 1200 mg/kg bw/day) on GD 10 and 11 (n = 23–31/group); 15, 22.5, or 30 mg/animal (600, 900, or 1200 mg/kg bw) on GD 10 (n = 12–32/group); or 15, 22.5, or 30 mg/animal (600, 900, or 1200 mg/kg bw) on GD 11 (n = 17–23/group). Results were compared to those obtained in negative controls dosed with vehicle on GD 6–17. Results for exposures occurring on GD 6–7 or GD 10–11 are summarized in Table 53. Effects on resorption and fetal weight were consistent with those observed after hydroxyurea exposure on GD 6–17. When hydroxyurea was administered on GD 10, adverse effects on resorption and fetal body weight were only observed at the high dose (30 mg/animal). No adverse effects on resorption or fetal weight were reported after exposure to hydroxyurea on GD 11. Incidences of each type of malformation observed after exposure to any of the time periods are summarized in Table 52. Malformations commonly observed after hydroxyurea treatment GD 6–7, 6, or 7 included cleft palates, sternum defects, encephaloceles, and vertebral defects. Most malformations observed on GD 6–7 occurred with exposure on GD 10–11, 10, or 11. In addition, limb and tail defects also occurred with exposure on GD 10–11, 10, or 11.

Based on these results, the study authors concluded that extreme caution is required when using hydroxyurea during pregnancy.

Strengths/Weaknesses: The authors conducted quite an extensive investigation with the dosing period on GD 6–17 in mice. The information provided is very useful for determining the effects of a 400 mg/kg bw/day dose level. The portion of the investigation with the dose levels above 400 mg/kg bw/day is less useful as are the instances in which hydroxyurea was administered on a single day of gestation, although the single-day studies provided critical period information. It is a weakness that the authors compare malformation data collected from cesarean section to those collected postnatally. Because the dam often will cannibalize malformed pups after they are born, the true incidence of postnatal malformations is unknown. In addition, the study did not have appropriate controls for certain comparisons. The data provide a LOAEL but not a NOAEL. The lack of visceral exams is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study has utility in that it provides dose-response information in mice for hydroxyurea exposures over a useful range of dose levels.

3.2.2.2 Parenteral exposure studies examining prenatal developmental toxicity: This section includes studies examining prenatal developmental toxicity and

Table 52
Summary of Hydroxyurea-Induced Malformations in Mice According to Dose and Exposure Day(s)

Endpoint	Treatment Dose (mg/animal) and Gestational Day										
	GD 6–17	GD 6–7		GD 10–11		GD 10			GD 11		
	10	15	30	15	30	15	22.5	30	15	22.5	30
Cleft palate[a]	—	3.0	23.7	8.0	28.7	1.7	6.0	19.3	—	0.9	16.1
Sternal defects	17.4	16.7	47.4	4.2	25.4	4.7	13.9	25.9	5.0	3.9	12.8
Encephalocele	12.5	—	15.8	0.4	9.8	0.4	—	—	1.5	—	0.6
Stump/no tail	2.0	—	2.6	8.4	23.8		13.9	23.0	—	—	5.6
Costal fusion	5.3	1.5	2.6	1.2	4.1	0.4	2.0	2.2	0.7	0.5	—
Vertebral defects											
Cervical fusion	5.9	6.1	7.9	—	4.9	0.4	—	—	—	—	—
Other fusion		4.5	—	—	—	—	2.0	—	—	—	—
Other defects											
Thoracic	7.9	—	15.8	13.3	56.5	2.1	26.7	45.1	—	—	17.3
Lumbar	1.3		10.5	5.3	27.0	—	6.0	32.5	0.7	—	5.3
Sacral	—	—	—	—	13.2	—	—	25.1	—	—	—
Polydactyly, hindpaw	—	—	—	—	2.4	—	1.0	—	—	3.9	5.6
Syndactyly, forepaw	—	—	—	1.2	3.2	—	—	0.7	—	9.5	20.1
Syndactyly, hindpaw	—	—	—	—	2.4	—	—	6.7	—	0.9	10.6
Tibial aplasia	—	—	—	—	9.8	—	—	7.4	—	—	—
Tibial shortening	—	—	—	—	4.9	—	—	—	—	—	1.2
Ulnar aplasia	—	—	—	—	—	—	—	1.5	—	—	0.6

From Roll and Bär (1969).
[a]Percentages of all malformations, apparently based on fetuses affected. Incidence for each type of malformation was $\leq 1.1\%$ in controls treated with vehicle on gestational day (GD) 6–17. When the columns were left blank by study authors, it was assumed that the malformation was not observed at that particular dose and day of exposure.

Appendix II

mechanisms of toxicity in mice exposed parenterally. Most studies included some examination of possible mechanisms of toxicity. Studies with dose-response information are presented first. Studies focusing on general observations of developmental toxicity are presented before studies examining only cellular-level mechanisms. Under each general category, studies are presented in order of publication.

Platzek and Schwabe (1999), support not indicated, examined prenatal toxicity in mice exposed to hydroxyurea alone and combined with 6-mercaptopurine. On GD 11 (GD 0 = 24 hr after the mating period), 13 NMRI mice were i.p. injected with saline vehicle and 7–8/group were i.p. injected with hydroxyurea **[purity not given]** at 250, 300, or 350 mg/kg bw. Dams were killed on GD 18. Fetuses were weighed and examined for gross and skeletal abnormalities. **[Statistical analyses were not conducted.]** Exposure to hydroxyurea had no significant effect on fetal viability, resorptions, fetal weight, or dam weight. Fetal abnormalities were increased in groups exposed to $\geq$300 mg/kg bw/day hydroxyurea, with incidence of abnormalities reported at 2.8% in controls, 21.6% in the 300 mg/kg bw group, and 43.4% in the 350 mg/kg bw group. Fused sternebra was the only abnormality observed in controls. The abnormalities most commonly observed in hydroxyurea-treated fetuses included skull defects, followed by forepaw oligo/syndactyly. The study authors identified a hydroxyurea NOAEL of 250 mg/kg bw/day. **[Based on the per-fetus total malformation data supplied in the study, the BMD_{10} is 213 mg/kg bw and the $BMDL_{10}$ is 188 mg/kg bw.]**

In a second study, 8–11 mice/group were s.c. injected with 16 mg/kg bw 6-mercatopurine, a highly teratogenic dose, and i.p. injected with 250 mg/kg bw hydroxyurea, the NOAEL dose, on GD 11. In one experiment, the two compounds were administered simultaneously. In two additional experiments hydroxyurea was given 2 hr before or 2 hr after administration of ^{35}S-6-mercatopurine. As in the previous study, it seems that dams were killed on GD 18. Results were compared to a study published previously on 6-mercatopurine and to the dose-response data for hydroxyurea described above. 6-Mercatopurine increased the incidence of hydroxyurea-induced skull malformations, especially when given before hydroxyurea. It was reported that hydroxyurea increased paw abnormalities when administered before 6-mercatopurine, and decreased paw abnormalities when administered after 6-mercatopurine.

In a study examining DNA modification, four pregnant dams were s.c. injected with 23.7 mg/kg bw ^{35}S-6-mercatopurine on GD 11. Two hours later, dams were i.p. dosed with 250 mg/kg bw hydroxyurea. A control group was exposed only to ^{35}S-6-mercatopurine. Embryos were dissected 4 hr after dosing with ^{35}S-6-mercatopurine, and incorporation of ^{35}S-thioguanine was measured by liquid scintillation counting. Compared to exposure to ^{35}S-6-mercatopurine alone, co-exposure to hydroxyurea reduced radiolabel uptake by 42%. The authors suggested that teratogenicity induced by 6-mercatopurine is at least due partly to DNA modifications.

Strengths/Weaknesses: This study presents a dose response relationship for hydroxyurea over a reasonable dose-range on GD 11. The inclusion of information on DNA synthesis is a strength. The authors provided a very good description of the different malformations that were observed. However, it is not clear if GD 11 is the most sensitive period for hydroxyurea-induced

malformations. The authors conducted skeletal exams but did not examine visceral organs for malformation. The lack of information about statistical analysis is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited utility in providing a dose-response information in mice for effects induced on GD 11, with a NOAEL (i.p.) of 250 mg/kg bw/day.

Seller and Perkins (Seller, 1983; Seller and Perkins, 1983), supported by the National Fund for Research into Crippling Diseases, examined the effects of prenatal hydroxyurea exposure on mutant curly-tail mice, a strain that is susceptible to neural tube defects. The most detailed information was included in Seller and Perkins (1983), whereas Seller (1983) reiterated some of the information for GD 9, one of the exposure days examined (the other exposure day was GD 8). Other compounds were also examined on GD 9 but will not be discussed here. The strain of mice used was CBA/Gr-ct/ct. GD 0 was considered the day of vaginal plug. Controls were treated with the saline vehicle on the appropriate gestation day and there was also a group of untreated dams. Mice were killed on GD 16. Implantation sites were examined, and embryos were sexed and assessed for developmental abnormalities. Mice displaying exencephaly, lumbosacral or caudal spinal bifida, or a curly tail were classified as having a neural tube defect. Exencephaly and spina bifida were classified as open lesions and curly or kinked tail were classified as closed lesions. Data were analyzed using a 2×2 contingency table.

On GD 8, mice were i.p. injected with hydroxyurea **[purity not given]** at 0, 200, 300, or 400 mg/kg bw. There were at least six dams in the control group and 8–12 dams in each treatment group. Numbers of embryos examined were 31 in the control group and 28–61 in the treatment groups. In the 400 mg/kg bw/day group compared to the vehicle control group, mean litter size was reduced (n = 2.3 vs. 5.2 in controls) and resorptions were increased (65 vs. 6% in controls). The differences did not appear to attain statistical significance. The incidence of neural tube defects was not altered by hydroxyurea dosing on GD 8; however, the proportion of open neural tube defects was significantly increased in the 400 mg/kg bw group (78 vs. 29% in controls). In the hydroxyurea groups, significant increases were observed for exencephaly alone (11% at 300 mg/kg bw, 50% at 400 mg/kg bw, 0 in controls) or exencephaly together with spina bifida and/or tail defects (i.e., both neuropores affected; 19% at 200 mg/kg bw, 28% at 300 mg/kg bw, 71% at 400 mg/kg bw, 0 in controls). Significant dose-related reductions in closure of the posterior neuropore were observed with hydroxyurea exposure (72% at 300 mg/kg bw, 29% at 400 mg/kg bw, 100% in controls). The only other abnormalities reported were gastroschisis occurring in 8% of embryos treated with 300 mg/kg bw and 36% of embryos treated with 400 mg/kg bw and a short stumpy tail in 7% of mice treated with 400 mg/kg bw.

On GD 9, mice were i.p. injected with hydroxyurea at 0, 200, 400, 500, or 600 mg/kg bw. There were 4–12 treated and at least 21 control dams/group. GD 9 was described as the day that final fusion and closure of the neural tube occur in curly tail mice. There were 120 fetuses examined in the control group and 25–67 fetuses in the treated groups. The percentages of fetuses with neural tube defects were significantly reduced by treatment with ≥ 400 mg/kg bw/day hydroxyurea (54% in controls, 27% at 400 mg/kg bw, 38% at 500 mg/kg bw, and 24% at 600 mg/kg bw); no dose response was observed. Exposure to hydroxyurea did not significantly affect percentages of embryos with open neural tube defects, exencephaly, or involvement of both neuropores. Involvement of the posterior neuropore was significantly reduced in groups treated with 400 and 600 mg/kg bw/day hydroxyurea (89% at 400 mg/kg bw/day, 83% at 600 mg/kg bw/day, 94% in controls). Hydroxyurea did not significantly affect the number of live embryos, mean litter size, or resorptions. The only other abnormality reported was gastroschisis in 3% of mice in the 400 mg/kg group and 5% of mice in the 500 mg/kg group.

Table 53
Prenatal Developmental Toxicity in Mice Orally Exposed to Hydroxyurea during GD 6–7 or 10–11

	Hydroxyurea dose, mg/animal (mg/kg bw)	
Endpoint	15 (600)	30 (1,200)
Exposure on GD 6–7[a]		
Pups delivered[b]	↓52%	↓70%
Early resorption	↑5.6-fold	↑4.5-fold
Midterm resorption	↑8.3-fold	↑33-fold
Fetal weight	↓15%	↓25%
Malformation	See Table 52	
Exposure on GD 10–11[a]		
Pups delivered	↔	↓39%
Midterm resorptions	↑7.7-fold	↑41-fold
Fetal weight	↓8%	↓15%
Malformation	See Table 52	

From Roll and Bär (1969).
[a]Results compared to controls exposed to vehicle on gestational day (GD) 6–17.
[b]The effect was not described as statistically significant by study authors, but due to the magnitude of change, the Expert Panel believes that the effect may be treatment related.
↑,↓, Statistically significant increase or decrease, respectively, compared to controls; ↔, no statistically significant effect compared to controls.

The study authors concluded that in curly tail mice, hydroxyurea can act as a teratogen when administered on GD 8 and a curative agent when administered on GD 9. It was hypothesized that ameliorative effects on GD 9 resulted from DNA inhibition.

Strengths/Weaknesses: Strengths are the use of multiple doses, permitting good dose-response data, two different days of administration, and adequate numbers of animals. The use of the curly-tail mouse is interesting, but the effect of hydroxyurea on this mutant is difficult to understand given the high background rate of neural tube defects.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation.

Woo et al. (2004), support not indicated, examined hydroxyurea-induced malformations in CD-1 mice. On GD 13 (GD 0 = day of vaginal plug), 10 mice/group were i.p. injected with hydroxyurea **[purity not given]** at 0 (distilled water vehicle), 400, or 800 mg/kg bw. Half the litters (5/group) were killed and evaluated on PND 0 (day of birth), and the other half were killed and

Table 54
Developmental Toxicity in Mice after Prenatal Exposure to Hydroxyurea and Evaluation at Postnatal Day 0 or 10 Weeks of Age

	Hydroxyurea dose (mg/kg bw/day)					
Endpoint	400	800	BMD_{10}[c]	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Evaluation at PND 0						
Body weight[a]	↓16%	↓18%	—	—	—	—
Cerebral cortex thickness[a]	↓22%	↓19%	—	—	—	—
Evaluation at 10 weeks of age						
Postweaning body weight gain[b]	↓	↓	—	—	—	—
Relative weight, males						
Brain	↓7%	↓11%	721	593	568	455
Lung	↓22%	↓15%	—	—	—	—
Left kidney	↔	↓7%	1207	793	1352	869
Spleen	↓12%	↓24%	340	294	439	366
Testis	↓12%	↓15%	532	426	671	521
Epididymis	↓40%	↓10%	—	—	—	—
Relative weight, females						
Brain	↓7%	↓9%	908	678	885	646
Lung	↔	↓9%	858	661	1011	822
Right kidney	↔	↓13%	707	584	739	616
Left kidney	↔	↓13%	787	694	788	697
Intestine	↓8%	↓8%	—	—	—	—
Ovary[d]	↔	↓25%	692	667	695	688
Uterus[d]	↔	↓27%	644	563	760	699
Kinked tail (0/53 in controls)	↔ (5/62)	↑ (9/50)	507	345	—	—
Microcephaly (0/53 in controls)	↑ (30/62)	↑ (25/50)		Models not satisfactory		
Cerebral cortex thickness[b]	↓	↓	—	—	—	—

From Woo et al. (2004).
[a]Value estimated by CERHR from graph.
[b]Percent changes compared to controls were not calculated because there appeared to be an error in presenting control values for males and females.
[c]Benchmark doses were only calculated for values with an evident dose-response relationship. For an explanation of the use of benchmark dose in this report, see footnote to Table 33.
[d]Benchmark doses estimated using polynomial model.
↑,↓, Statistically significant increase or decrease, respectively, compared to controls; ↔, no statistically significant effect compared to controls.

Appendix II

evaluated at 10 weeks of age. On PND 0, pups were weighed and examined for external malformations. Half of the pups were evaluated for visceral malformations, and the other half were examined for skeletal malformations. **[Based on information provided in the results section, it appears that malformations were actually evaluated in the 10-week-old offspring.]** In the study with the 10-week evaluation, offspring and dams were separated at 4 weeks of age. Offspring were weighed every 2 weeks and killed at 10 weeks of age. Organs, including ovaries, testes, uteri, and epididymides, were weighed and fixed in 10% neutral buffered formalin for histopathologic evaluation. Statistical analyses included Student's or Welch *t*-test.

In groups evaluated during either period, there were no significant differences in total number of litters, litter size, or number of dead pups. Effects observed with hydroxyurea exposure are summarized in Table 54. In offspring from hydroxyurea-exposed groups, body weights were lower on PND 0 and during the postweaning period. Relative weights (to body weight) of several organs were lower in the hydroxyurea than control groups, as described in Table 54. Anomalies consisting of micrencephaly, hydrocephalus, and curved coccygeal vertebrae were increased in both dose groups at 10 weeks of age and were more severe in the high dose group. Thickness of the cerebral cortex was reduced in both dose groups evaluated at either time period. No other histopathologic changes were observed. Based on the results of this and previous studies from the same laboratory, the study authors proposed that apoptosis may be involved in developmental abnormalities associated with hydroxyurea exposure.

Strengths/Weaknesses: The use of multiple dose levels is a strength, but the doses were too high and the number of mice was too low. The use of a single day of treatment and the lack of a NOAEL are weaknesses. The mice were examined postnatally for evidence to support a mechanism to explain reduced growth rates; however, the study did not control for any instances of cannibalism, and therefore may have missed terata that would have been observed at these high dose levels. This study has limited utility in this evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Yan and Hales (2005), supported by the Canadian Institutes of Health Research, examined the role of oxidative stress in redox-sensitive transcription factors in hydroxyurea-induced developmental toxicity in mice. CD-1 mice were i.p. injected with saline vehicle or hydroxyurea **[purity not given]** at 400, 500, or 600 mg/kg

Table 55
Developmental Toxicity Observed in Mice Exposed to Hydroxyurea by Intraperitoneal Injection on GD 9

	Hydroxyurea dose (mg/kg bw)		
Endpoint	400	500	600
Fetal death rate, GD 18[a]	↔	↑ (~43 vs. 5% in controls)	↑ (~53 vs. 5% in controls)
External malformation rate, GD 18[a]	↔	↑ (22.2 vs. ~2% in controls)	↑ (87.7 vs. ~2% in controls)
Skeletal malformation rate, GD 18[a]	↔	↑ (~35 vs. 5% in controls)	↑ (90.2 vs. ~5% in controls)
AP-1 C-FOS binding activity, GD 9 embryos	↑ 3-fold at 3 hr after exposure	↑ 4-fold at 3 and 6 hr after exposure	↑ 4-fold at 3 and 6 hr after exposure
Glutathione level in embryo	↔	↓ 6% at 3 hr	↓17% at 3 hr and 12% at 6 hr

From Yan and Hales (2005).
[a]All or some values estimated by CERHR from a graph.
↑,↓, Statistically significant increase or decrease, respectively, ↔, no significant effects compared to controls.
GD, gestational day.

bw on GD 9 (GD 0 = day of mating). At 0.5, 3, or 6 hr after injection, 7–10 dams/treatment group were killed, and transcription factor activities and oxidative stress were evaluated. Glutathione homeostasis was determined in embryos, yolk sacs, and maternal liver. Activator protein-1 (AP-1) DNA-binding sites were evaluated in fetuses and yolk sacs using electrophoretic mobility shift assay. Binding activities of AP-1 c-Fos heterodimer, AP-1 c-Jun homo/heterodimer, NF-κB p50 dimer, and NF-κB p56 dimer were examined in embryos and yolk sacs using enzyme-linked immunosorbent assay (ELISA) techniques. Expression of c-Fos in embryos was examined using an immunohistochemistry technique. On GD 18, 8–10 dams/group were killed. Implantation sites were examined, and fetuses were evaluated for viability and external malformations. Two normal and two malformed fetuses were selected randomly from each litter for an examination of skeletal malformations. Statistical analysis included ANOVA followed by post-hoc Holm-Sidak test.

Developmental toxicity observed in GD 18 fetuses is summarized in Table 55. Exposure to hydroxyurea at ≥500 mg/kg bw resulted in increased fetal death (i.e., resorption) and external and skeletal malformations. The types of malformation commonly observed included curly tail and hindlimb defects characterized by oligodactyly (missing digits), hemimelia (total or partial absence of distal limb), and amelia (absence of limb). In most cases, malformations were observed on one side of the body. Short ribs were also observed. On GD 9, AP-1 binding activity was increased in yolk sac and embryos at 3 hr after exposure. ELISA techniques were used to further characterize the type of AP-1 family members expressed. Results are summarized in Table 55. The activity of AP-1 c-Jun was not increased, but binding activity of AP-1 c-Fos was increased in embryos at all doses. Binding activity of NF-κB p50 and p56 dimers was also examined and found to be unaffected in GD 9 embryos or yolk sacs after exposure to hydroxyurea. In studies to localize c-Fos immunoreactivity, it was observed that exposure to 600 mg/kg bw hydroxyurea dramatically increased c-Fos expression in hindbrain, neural tube near the caudal region, blood cells, dorsal aorta, branchial arch, and atrial and ventricular walls of the heart, all areas in which c-Fos was expressed in control animals. Although decreases in embryo glutathione content were observed at the mid and high doses, an evaluation of oxidative stress by assessing glutathione homeostasis showed no effect of hydroxyurea treatment on the ratio of oxidized to reduced glutathione in maternal liver, embryos, or yolk sacs on GD 9. The study authors concluded that induction of AP-1 DNA-binding activity in mouse embryos is a sensitive marker of hydroxyurea-induced toxicity.

Strengths/Weaknesses: The use of multiple doses, permitting evaluation of a dose-response relationship, is a strength. The study design explored whether hydroxyurea caused teratogenicity through induction of oxidative stress and resultant changes in transcription factors. Based on a limited number of the fetuses used for the skeletal exam, a dose-response relationship was reported for resorptions and malformations. However, interpretation is difficult because changes in AP-1 induction occurred at a dose level that was reported as a NOAEL for malformation rates. The lack of effect of 400 mg/kg bw/day on GD 9 is slightly different from what others have reported. Therefore, the question arises whether the authors examined a transcription factor that is directly or even indirectly related to malformations. Overall, this study is an interesting mechanistic study.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in demonstrating a NOAEL of 400 mg/kg bw and a LOAEL of 500 mg/kg bw in mice treated on GD9.

Kwasigroch and Skalko (1985), supported by NIH, examined the effects of prenatal hydroxyurea exposure alone and in combination with 5-bromodeoxyuridine on malformations in mice. On GD 11 (GD 0 = day of vaginal plug), ICR mice were i.p. injected with 250 mg/kg bw hydroxyurea **[purity not given]** in distilled water or 500 mg/kg bw 5-bromodeoxyuridine. Additional groups of mice were treated with the same doses of hydroxyurea and 5-bromodeoxyuridine administered simultaneously or 1 or 3 hr apart. A control group was left untreated. Ten dams from each dose group were killed on GD 17. Fetuses were examined for cleft palate and external malformations. Data were analyzed by χ^2 test. Seven dams/group were killed on GD 12. Forelimbs and hindlimbs from 1 embryo/litter were pooled and cultured for 6 days. The cultured limbs were analyzed using histopathologic and imaging techniques. Statistical analyses for limb-bud histopathology data included ANOVA and Newman-Keuls test.

Exposure to hydroxyurea alone increased the incidence of cleft palate to 2.4% compared to 0 in the control

but did not increase incidences of resorptions or limb malformations. Exposure to 5-bromodeoxyuridine alone increased the incidence of cleft palate to 22.9%, but the incidence was decreased to 3.6% with co-administration with hydroxyurea. Digit defects such as hindlimb syndactyly and fore- and hindlimb ectrodactyly, which were not observed after treatment with hydroxyurea or 5-bromodeoxyuridine alone, were observed when the two compounds were co-administered. The highest incidences of malformations were observed with increased time interval between hydroxyurea and 5-bromodeoxyuridine exposure.

In cultured limbs obtained from embryos exposed in utero to hydroxyurea, there were no observations of fused or missing paw parts in forelimb or hindlimb. Co-treatment with hydroxyurea and 5-bromodeoxyuridine increased the incidence of fused paws in hindlimbs and missing paw parts in forelimbs and hindlimbs that were observed with exposure to 5-bromodeoxyuridine alone. Image analysis studies showed some differences in bone area after hydroxyurea treatment but no consistent changes in shape or form of limbs. Some changes in long bone-to-paw ratios were observed with co-exposure to hydroxyurea and 5-bromodeoxyuridine. The study authors concluded that co-exposure to hydroxyurea and 5-bromodeoxyuridine resulted in a teratogenic response that differed from that observed with exposure to either compound alone.

Strengths/Weaknesses: This study reports an in-depth mechanistic investigation into hydroxyurea using a reasonable dose level. However, the study was limited to a single dose on a single day of gestation and it is not clear if different mechanisms may occur on other periods of development or at different dose levels. For the in vitro work, the authors carried out a statistical analysis using the fetus (not the litter) and therefore did not consider litter effects.

Utility (Adequacy) of CERHR Evaluation Process: This study is not useful in the evaluation.

Sadler and Cardell (1977), supported by NIH, examined ultrastructural alterations in mouse embryos exposed to hydroxyurea. ICR/DUB mice were i.p. injected with 250 or 500 mg/kg bw hydroxyurea **[purity not given]** in saline vehicle on GD 9 (plug = GD 1). The 250 mg/kg bw dose had been shown previously to be teratogenic, and the 500 mg/kg bw dose had previously been shown to be embryolethal. Controls were untreated. Mice were killed and embryos were removed between 15 min and 4 hr after hydroxyurea exposure. Embryos were sectioned, and neuroepithelial cells from the cranial region of the neural folds were examined by light and electron microscopy. **[Numbers of dams treated and embryos examined were not reported.]** At 1–2 hr after exposure to either hydroxyurea dose, necrotic cells (characterized by pyknosis) were scattered throughout neuroepithelial tissues, and numbers of mitotic figures decreased. By 4 hr after exposure, mitotic cells were observed only occasionally. Ultrastructural observations in neuroepithelial cells from hydroxyurea-exposed animals included a breakdown of polysomes into ribosomes and condensed nucleoli at 15 min to 1 hr after exposure. At 1–2 hr after exposure, the cytoplasm was condensed, cells became distorted or fragmented, dilated vesicles appeared, and heterochromatin clumps were observed in the nucleus. Observations at 2–4 hr after exposure included extremely condensed cytoplasm, fragmentation, phagocytosis, and destruction of necrotic cells. The study authors concluded that substances adversely affecting the nucleus induce a type of necrosis that is similar to physiological cell death found in some developing tissues.

Strengths/Weaknesses: In this investigation, the researchers used hydroxyurea to affect neuroepithelial cells on GD9 in mice. The study used two dose levels, one that caused exencephaly and one that caused embryo lethality. Strengths are the use of histology and ultrastructural examination hr after dosing. Weaknesses are the lack of evaluation of malformations and the lack of details on dose-response relationship, including a lack of a NOAEL.

Utility (Adequacy) for CERHR Evaluation Process: This study shows rapid effects of hydroxyurea on proliferating tissues, but is of limited utility for a quantitative evaluation.

Herken et al. (1978), supported by the German Research Council, examined the effects of hydroxyurea on the cell cycle and development of necrosis in the CNS of mouse embryos. Two sets of NMRI mice were exposed to hydroxyurea **[purity not given]**. One set of NMRI mice **[number not reported]** received an i.v. injection of 500 mg/kg bw hydroxyurea on GD 10+7 hr (GD 0 = day of fertilization **[0 hr not specified]**). Groups of mice were killed every 30 min until GD 10+17 hr. Embryos were sectioned and examined by light and electron microscopy. A second set of four mice were i.p. injected with 10 mCi ^{3}H-thymidine/kg bw on GD 10+7 hr. Three of the mice were also exposed to 500 mg/kg bw hydroxyurea during the same time period. Mice were killed 4 hr after exposure, and embryos were sectioned and subjected to autoradiography. Examination by light microscopy showed necrosis in the intermediate zone of the neuroepithelium of the brain anlage, which was first observed at 2 hr after hydroxyurea exposure and which reached its maximum value at 10 hr after exposure. Nuclear pyknosis, shrinkage, and fragmentation were observed in nearly every second cell of the intermediate zone. Single necrotic cells were observed in the paraventricular zone, and only sporadic mitoses were observed in the layer near the ventricle. Examination by electron microscopy showed chromatin condensation in nuclei and nucleoli shrinkage 2.5 hr after exposure. Also observed were cytoplasm shrinkage, clustering of cellular organelles, development of coarse cell processes, and disintegration of polysomes into single ribosome. By 3 hr after exposure, the number of cells displaying these changes increased and cells began breaking down. Cell fragments were observed in the extracellular space, but most were phagocytosed by neighboring cells. Autoradiography showed lower density of cell labeling in hydroxyurea-exposed than in control embryos. About 98% of necrotic cells in the CNS were labeled. Because thymidine is incorporated mainly during the S-phase, the study authors concluded that hydroxyurea influences only metabolic processes that occur during the S-phase.

Strengths/Weaknesses: This study reports the visual evidence of some of the cellular effects of hydroxyurea. However, the dose level of hydroxyurea was very high and was administered i.v. on a single day of gestation. The ^{3}H-thymidine labeling to show that it was proliferating cells that had died is a strength.

Table 56
Autoradiographic Results in Mouse Spinal Cord Cells After Prenatal Hydroxyurea Exposure

		Hours after exposure						
Condition	Control	1.5	2	2.5	4	6	8	10
Necrosis	0.1±0.09	0.4±0.26	1.55±1.51	17.47±0.45	23.34±10.52	24.77±0.59	37.19±0.16	44.8±0.67
Mitosis	8.06±0.84	8.16±0.21	8.1±2.68	3.03±0.63	3.05±0.48	0.19±0.06	0.61±0.06	8.48±0.39
Labeled	27.01±2.68	0	0	0	0	22.6±1.3	30.19±0.5	36.48±0.44
Unlabeled	64.82±3.35	91.43±0.42	90.35±1.55	79.5±10.43	73.6±10.43	52.42±1.21	32.01±0.48	10.22±0.19

From Herken (1980).
Values presented as mean±SD percent of cells.

Table 57
Effects in Neuroepithelial Cells of the Spinal Cord of Mouse Embryos After Exposure to Hydroxyurea Alone or in Combination with Deoxycytidine Monophosphate

Treatment (mg/kg bw)						
Hydroxyurea	Deoxycytidine	Necrosis	Labeled necrosis	Mitosis	Labeled mitosis	Labeling index
0	0	0.1±0.06	—	7.64±0.56	—	35.0±0.25
500	0	29.44±3.78	6.43±4.55	0.11±0.06	—	26.7±3.24[b]
500	500	31.9±7.47	5.45±1.48	0.35±0.14	—	18.5±1.4[b]
500	700	13.95±4.65	6.15±4.31	1.14±0.72	34.5±13.44	49.8±4.95
500	800	15.25±13.25	3.5±1.24	0.89±0.31	33.3±11.71	41.65±3.74
500	900	20.13±10.58	9.13±4.07	0.81±0.8	30.57±26.79	37.8±9.3

From Herken (1984).
[a]Data presented as mean±SD percent of cells.
[b]In one embryo.

Utility (Adequacy) of CERHR Evaluation Process: This study is of limited utility given the dose level used and the limited dosing regimen.

Herken (1980), supported by the German Research Council, examined the effects of hydroxyurea exposure on the cell cycle of spinal cord cells of fetal mice. On GD 10+3 hr (GD 0 = day of fertilization **[0 hr not specified]**), NMRI mice received an i.v. injection of 500 mg/kg bw hydroxyurea **[purity not given]**. Mice were i.p. injected with 5 mCi/kg bw ^{3}H-thymidine 45 min before they were killed at 1, 1.5, 2, 2.5, 4, 6, 8, and 10 hr after hydroxyurea exposure. Embryos were removed, sectioned, and examined by autoradiography. Two dams were sacrificed at each time period, and two embryos/dam were examined. Necrosis, mitosis, and labeled cells were determined at the level of attachment of the upper limb. Total numbers of cells were counted for each section. Percentages of cells undergoing necrosis, mitosis, and labeling are summarized in Table 56. Necrosis was first observed at 1.5 hr after hydroxyurea treatment and increased with time. Decreases compared to controls in mitotic index were first observed 2.5 hr after exposure, and the index remained lower than the control value until 10 hr after exposure. Labeled cells were first observed at 6 hr after treatment. The unlabeled cells were said to be in G_1 or G_2 phase. Changes in patterns of necrotic cells were observed over the time course of the study. At 6–8 hr after treatment, necrotic cells were dispersed among labeled cells. Ten hour after treatment, necrotic cells were localized in the periphery of the spinal cord and labeled cells were adjacent to the lumen. Based on the pattern of effects observed, the study authors stated that hydroxyurea damaged cells in S-phase and that those cells became necrotic. They concluded that only cells entering S-phase after the hydroxyurea exposure could proceed with normal DNA replication.

Strengths/Weaknesses: This study suggests an effect of hydroxyurea on cell cycle and, therefore, a time of greatest sensitivity during the cell cycle. However, the use of a single high intravenous dose on 1 day of gestation is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Herken et al. (1982), supported by the German Research Council, conducted a study to determine the effects of hydroxyurea on cell kinetics and necrosis in the mouse embryonic spinal cord. On GD 10 (GD 0 = day of vaginal plug), 15 NMRI mice/group were i.p. injected with 500 mg/kg bw hydroxyurea **[purity not given]**, 500 mg/kg bw hydroxyurea+1 mg/kg bw colchicine, or 1 mg/kg bw colchicine. Three untreated animals were used as controls. Three animals/group were killed at 1.5, 1.83, 2.17, 2.5, and 4 hr after hydroxyurea exposure. Embryonic spinal cord sections were prepared for quantification of mitotic and necrotic neuroepithelial cells.

The mitotic index was 7.7% in control cells. Hydroxyurea reduced the mitotic index to 4.0% at 1.5 hr after exposure and to ≤0.69% during the next hour and for the remainder of the 4-hr evaluation period. **[Slightly different numbers were provided in the study abstract for percent reduction in mitotic index; the numbers used in this summary were obtained from the main body of the report.]** Necrosis was first observed in the region of the prospective alar plate at 1.83 hr after hydroxyurea exposure. The percentages of necrotic cells

were measured at 5.12–34.38% between 2.5 and 4 hr after exposure. With co-administration of hydroxyurea and colchicine, the decrease in mitotic rate was similar to that observed with exposure to hydroxyurea alone. Few necrotic cells were observed after co-administration of colchicine and hydroxyurea. A change in nucleus shape from oval to round and condensed chromatin were observed with exposure to colchicine alone and colchicine in combination with hydroxyurea. Based on the timing of observations, the study authors concluded that hydroxyurea likely blocks the transition of neuroepithelial cells during the S/G_2 phase. Based on results observed with colchicine co-treatment, the study authors postulated that different mechanisms are responsible for cell necrosis and changes in cell kinetics.

Strengths/Weaknesses: This study used an inhibitor of microtubule and microfilaments to investigate the mechanistic pathway of hydroxyurea effects on embryonic spinal cord. The use of a single high intravenous dose level on a single day of gestation is a weakness. The effects observed at discrete time points are difficult to interpret as cell cycle delay or arrested mitosis will be changing as the levels of the two agents change over time.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility.

Herken (1984), supported by the German Research Council, examined the effect of deoxycytidine monophosphate on hydroxyurea-induced cytotoxicity in the mouse embryonic spinal cord. On GD 10 (GD 0 = day of vaginal plug), three NMRI mice/group were i.p. injected with 500 mg/kg bw hydroxyurea **[purity not given]** alone or in combination with 500, 700, 800, or 900 mg/kg bw deoxycytidine monophosphate. Three hours later, mice were i.p. injected with 5 mCi/kg bw ^{3}H-thymidine and killed 1 hr later. Embryos were sectioned and subjected to autoradiography for examination of mitosis, necrosis, and labeled cells in neuroepithelium of spinal cord region C7/C8. Data were analyzed by Student's *t*-test. Results of the study are summarized in Table 57. **[Although the author discussed statistical significance of some effects, it was not clear if all statistically significant effects were identified.]** Exposure to hydroxyurea resulted in increased necrosis and decreased mitosis in spinal cord neuroepithelial cells. Exposure to 700 mg/kg bw deoxycytidine monophosphate in combination with hydroxyurea resulted in a significant decrease in necrosis compared to exposure to hydroxyurea alone. An increase in mitotic index was reported for co-exposure to hydroxyurea and 800 mg/kg bw deoxycytidine monophosphate compared to exposure to hydroxyurea alone. No significant effects were reported for co-exposure to 900 mg/kg bw deoxycytidine monophosphate. The study author concluded that optimal inhibition of hydroxyurea-induced cytotoxicity occurred with co-exposure to 700 mg/kg bw deoxycytidine monophosphate.

Strengths/Weaknesses: The authors used a DNA base to investigate the mechanism of cellular site of action of hydroxyurea. The use of a single high i.v. dose on a single day of gestation and the lack of evaluation of malformations are weaknesses. Although the mechanism of action suggested by this research may be relevant for hydroxyurea at the dose level used in this study, it is unclear if the same mechanism applies to lower dose levels that cause teratogenicity.

Utility (Adequacy) for CERHR Evaluation Process: This study provides further support for a cell cycle/cell death mode of action but is of limited utility in a quantitative evaluation.

Herken (1985), supported by the German Research Council, examined ultrastructural changes in the neural tube of mouse embryos after exposure to hydroxyurea, with and without co-exposure to colchicine. On GD 10 (GD 0 = day of vaginal plug), NMRI mice received 500 mg/kg bw hydroxyurea **[purity not given]** in saline vehicle (n = 24/group) or 500 mg/kg bw hydroxyurea + 1 mg/kg bw colchicine (n = 48/group). A control group of 6 mice was left untreated. **[Additional mice were exposed to colchicine alone but that portion of the study will not be addressed. It is presumed that exposure to hydroxyurea was by i.p. injection, consistent with colchicine exposures and other reports from this author, but administration route was not specified.]** Mice were killed at 30-min intervals between 0.5 and 4 hr after exposure. Three embryos/time period in the hydroxyurea group, six embryos/time period in the hydroxyurea + colchicine group, and six embryos in the control group were sectioned for examination of the neural tube by electron microscopy. At 90 min after hydroxyurea exposure, chromatin condensation was observed in nuclei of neuroepithelial cells in the intermediate zone of the lateral alar plate of the spinal cord. Soon after, a breakdown of polysomes into ribosomes was observed. Four hours after exposure, numerous neuroepithelial cells in the spinal cord were necrotic. No necrosis was observed when colchicine was simultaneously administered with hydroxyurea. The study authors hypothesized a mechanism for cell death in which exposure to hydroxyurea inhibited DNA synthesis, which led to activation of cytoskeletal element in the nucleus, changes in chromatin distribution resulting in attachment of chromatin to the nuclear membrane, and ultimately nuclease release and attack on DNA.

Strengths/Weaknesses: This study appears to duplicate Herken et al. (1982).

Utility (Adequacy) of CERHR Evaluation Process: This study is not useful in the evaluation because it appears to be a duplicate of another publication.

Woo et al. (2003), support not indicated, evaluated apoptosis in offspring of hydroxyurea-treated treated pregnant mice. Hydroxyurea **[purity not given]** 400 mg/kg bw was given i.p. to 30 pregnant Crj:CD-1 (ICR) mice on GD 13 **[GD 0 not defined]**. Groups of 5 dams were killed 1, 3, 6, 12, 24, and 48 hr after the treatment, and five fetuses/dam were processed for standard light microscopy and for terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining of sections of brain, lung, mesenchyme (renal, craniofacial and limb), liver, kidney, and alimentary tract. Electron microscopy was carried out to show that ultrastructural changes in pyknotic cells represented apoptosis. Comparisons were made to fetuses from two dams given i.p. distilled water. The number of TUNEL-positive cells/mm^2 brain was evaluated on a per dam basis using Student's *t*-test. The assessment of pyknotic cells per section was made semi-quantitatively. Pyknosis in fetal tissues was first observed 3 hr after treatment and

peaked in brain 12 hr after treatment. Pyknotic cells appeared in the brain first in the middle layer of the ventricular zone and by 12 hr involved the middle and dorsal layers. The number of TUNEL-positive cells paralleled the semi-quantitative assessment of pyknotic cells, with a peak at 12 hr in the brain. Pyknotic and apoptotic cell estimations had returned to baseline values by 48 hr. The pattern of pyknotic and apoptotic cells in the fetal lung was similar to although less prominent than that in brain. The authors concluded that excess apoptotic cell death in the CNS and other tissues may underlie the developmental toxicity of hydroxyurea.

Strengths/Weaknesses: The strength of the study is that it shows clearly that hydroxyurea causes apoptosis in target tissues. The weakness is that not much else was done. Hydroxyurea could also affect the cell cycle (proliferation), and this effect might occur at a lower dose than does apoptosis.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this study is limited in showing only one facet of pathogenesis.

Woo et al. (2005), support not indicated, examined the effect of hydroxyurea exposure on apoptosis in mouse fetal lung. On GD 13, CD-1 mice were i.p. injected with distilled water or 400 mg/kg bw hydroxyurea **[purity not given]**. Six control dams and eight treated dams/time period were killed at 1, 3, 6, 12, 24, or 48 hr after exposure. During each time period, three control dams and five treated dams were injected with bromodeoxyuridine before being killed for histopathology, immunochemistry, and reverse transcriptase-polymerase chain reaction (RT-PCR) analyses of fetuses. TUNEL assay was used for the immunohistochemical detection of fragmented DNA. Immunohistochemistry methods were also used for detection of bromodeoxyuridine, p53, and cleaved caspase 3. For RT-PCR analysis, total RNA was isolated from lungs of seven fetuses/dam. Flow cytometry analyses to measure sub-G_1 DNA content were conducted in offspring from three dams/group at each time period. Statistical analyses included Student's and Welch *t*-tests.

In fetuses from the hydroxyurea group, pyknotic cells that stained positive for TUNEL and cleaved caspase 3 were observed primarily among mesenchymal cells. The number of TUNEL-positive cells began increasing at 3 hr, peaked at 6 hr, and then decreased over the remainder of the 24-hr period after exposure. A large increase in p53-positive cells was observed at 1 and 3 hr after hydroxyurea exposure; p53-positive cell numbers decreased rapidly after 3 hr, and reached control levels 24 hr after exposure. Bromodeoxyuridine-positive lung cells were decreased at all time periods except at 6 hr after hydroxyurea exposure. Changes in expression of apoptosis-related genes in the hydroxyurea group included increased messenger RNA (mRNA) for *p21*, *bax*, and cyclin G. An increase in expression of *fas* mRNA was reported but was not statistically significant. Effects observed by flow cytometry in the hydroxyurea group included initial increases of sub-G_0/G_1 and S-phase fractions at 3 hr after exposure and decreases at 6 hr or more after exposure. A drastic decrease in G_2/M fraction occurred at 3 hr after exposure in the hydroxyurea group. The study authors interpreted the flow cytometry results as suggesting arrested cell cycle in the S-phase at 3 hr after exposure. The study authors stated that hydroxyurea-induced apoptosis in mouse fetal lung may be related to p53 induction.

Table 58
Major Findings in Cats Dosed with Hydroxyurea During Pregnancy

	Hydroxyurea dose (mg/kg bw/day)		
Endpoint	0	50	100
Mated cats	17	17	17
Cats that aborted	2	1	1
Cats that were killed	0	0	2
Cats not pregnant	5	4	10
Cats with 100% resorptions	3	3	3
Cats with live fetuses	7	8	1
Total live fetuses	40	38	2
Dead fetuses	3	0	0
Resorptions	20	16	13[a]
Mean ± SE weight (gm)	11.8 ± 0.03	11.3 ± 0.4	9.7 ± 0.9[a]
Litters with malformed fetuses/litters examined	2/7	5/8	1/1
Malformed fetuses/fetuses examined	4/40	11/38	1/2
Fetuses with visceral malformations/fetuses examined	1/19	6/17	1/1
Fetuses with skeletal malformations/fetuses examined	3/21	5/21	0/1

From Khera (1979).
[a] $p < 0.05$.

Strengths/Weaknesses: These data suggest an increased incidence in apoptosis within the fetal lung by the use of expression levels of target genes involved in apoptosis. The relevance of this mechanism to other days of gestation or other dose levels remains to be determined. The use of a single high dose level on a single day of gestation limits the extrapolation to other time periods. However, these findings give credence to what has been observed in the histopathologic studies.

Utility (Adequacy) for CERHR Evaluation Process: This interesting mechanistic study is useful in the evaluation.

3.2.3 Cat. Khera (1979), support not indicated, examined developmental toxicity in offspring of cats exposed to hydroxyurea. The effects of sodium diphenylhydantoin were also examined but will not be discussed. Short-haired cats of European, Persian, or random descent (n = 17/group) were orally exposed to hydroxyurea **[purity not given]** in gelatin capsules at 50 or 100 mg/kg bw/day on GD 10–22. A group of 17 control cats was given empty capsules during the same time period. Cats were killed on GD 43, and fetuses were examined for skeletal and gross visceral malformations. **[No details were provided regarding methods for fetal examination.]** The litter was considered the experimental unit in data analyses, which were conducted by Bonferroni *t*-test. Reported maternal and fetal effects are summarized in Table 58. At the high dose, maternal weight gain **[data not shown by authors]** and pregnancy rate were reduced. Significant effects in fetuses of the high-dose group included increased resorptions and decreased fetal weights. In the only high-dose cat with live fetuses, cyclopia was observed in one of two fetuses.

The apparently increased incidence of fetal malformations in the low-dose group did not attain statistical significance. The study authors noted that borderline significance would have been attained for the low-dose group if data were included from one control cat and one low-dose cat that had been removed from the study due to threatened abortion. Cleft palate and microphthalmia were the types of malformation observed most frequently in the low-dose group. Other types of malformations observed at lower incidences included exencephaly, microcephaly, split eyelids, rudimentary kidneys, ectrodactyly, hindlimb micromelia, missing tail, and fused ribs and vertebrae. The study author concluded that this study showed weak teratogenic activity of hydroxyurea in cats.

Strengths/Weaknesses: An appropriate number of animals was used. The test chemical was defined, but the purity was not stated. There was no reported chemical verification of dosing preparations. The gestational age of animals and duration of exposure were appropriate. Appropriate endpoints were observed, but methods were not detailed. Data were reported in appropriate detail.

Utility (Adequacy) for CERHR Evaluation Process: This study has some utility in the evaluation process. Results suggest a teratogenic effect of exposure to hydroxyurea, but the lack of a statistically significant effect makes this a ''no effect'' study outcome.

3.2.4 Rabbit. Studies in rabbits, conducted with parenteral exposures, examined general developmental toxicity induced by hydroxyurea and possible mechanisms for developmental toxicity. Most studies reported developmental toxicity effects and examined possible mechanisms of toxicity. The studies are presented in order of publication.

DeSesso and Jordan (1977), supported by the Medical College of Virginia Foundation and NIH, examined the effects of prenatal hydroxyurea exposure in rabbits. On GD 12 (GD 0 = day of mating), 7–13 New Zealand White rabbits/group were s.c. injected with 750 mg/kg bw hydroxyurea **[purity not given]** in distilled water or saline or were left untreated. Rabbits were killed on GD 29. Resorption sites were examined, and fetuses were weighed and examined for gross and skeletal malformations. In a separate study, embryos obtained at 2–32 hr after exposure of rabbits to 750 mg/kg bw hydroxyurea on GD 12 were examined histologically. The effects of methotrexate and acetazolamide were also examined but will not be discussed here. Data were analyzed by Student's *t*-test. Exposure to hydroxyurea resulted in a 100% malformation rate compared to 4–6% malformation rates in both control groups. Hydroxyurea-induced malformations included cleft lip, cleft palate, micrognathia, internal hydrocephalus, ectopic kidney, generalized body edema, stunted tail, and severe limb anomalies. Malformations that were occasionally observed with hydroxyurea treatment included encephalocele, microcephaly, hindlimb phocomelia, syndactyly, hydroureter, and hydronephrosis. Bone defects were consistent with external malformations; additional skeletal defects included defects in ribs, vertebrae, and facial bones. A significant reduction in fetal weight **[by ~30% from controls]** was also observed in the hydroxyurea group. The resorption rate in the hydroxyurea group was apparently higher than controls (61 vs. 10–13%), but the effect was not reported to be statistically significant. Histologic evaluation of hydroxyurea-exposed embryos showed dense basophilic intercellular granules resembling pyknotic nuclei in limb-bud mesenchyme, neural tube, and dorsal root ganglia. The granules were first observed 4 hr after treatment, and numbers continued to increase for up to 16 hr after treatment. Few mitotic figures were observed. No repair was observed at 32 hr after exposure. The study authors noted a possibility that forelimb malformations induced by hydroxyurea resulted from cell death in limb-bud mesenchyme.

Strengths/Weaknesses: An appropriate number of animals was used. The test chemical was defined, but the purity was not stated. There was no reported chemical verification of dosing preparations. The gestational age of animals and duration of exposure were appropriate. Appropriate endpoints were observed, and the endpoints were observed at appropriate life stages. The histologic methods were good. Data were reported in appropriate detail, and appropriate statistics were used. Use of one dose and one developmental stage at treatment are weaknesses.

Utility (Adequacy) of CERHR Evaluation Process: This study is useful in the evaluation process and shows teratogenic effects of exposure to hydroxyurea in rabbits. Mechanistic support is also provided by this study, which showed cell death in susceptible organs.

Millicovsky and DeSesso (1980), supported by the American Heart Association and the Orthopaedic Research and Education Foundation, examined cardiovascular alterations in rabbits exposed to hydroxyurea during prenatal development. New Zealand White rabbits were randomly assigned to groups and were s.c. injected on GD 12 (GD 0 = day of mating) with hydroxyurea **[purity not given]** 750 or 500 mg/kg bw. Negative controls were left untreated or exposed to saline solutions with osmolarities ranging from 300–4000 mOsm and pH of 4.5–9.0. Rabbits were anesthetized, and direct in vivo microscopic observation of the fetal cardiovascular system was carried out every 1 min during the first 10 min after treatment and every 5 min during the remainder of the 60-min period after treatment. Cardiovascular observations included alterations in heart rate, condition of the pericardial cavity, and changes in embryonic blood vessels. A total of 134 embryos from 23 litters were examined in the negative- and saline-control groups. Ninety-seven embryos from 16 litters were studied in the 750 mg/kg bw group, and 30 embryos from three litters were studied in the 500 mg/kg bw group. Representative embryos from all groups were examined histologically.

No adverse structural or functional effects were observed in embryos of the control groups. Alterations observed in embryos of the 750 mg/kg bw group included anterior cardinal vein dilation within 4 min and craniofacial hemorrhage, pericardial hemorrhage, and cardiac tamponade within 4–9 min after exposure. Alterations in craniofacial microvasculature affected 90% of embryos within 4 min after exposure. Periocular hematomas and hemorrhage within cerebral ventricles were observed in >40% of embryos. Cardiac tamponade occurred in 45% of embryos. Histopathologic observations in the 750 mg/kg bw group included endothelial discontinuities and extravasation of blood cells into the mesenchyme of the craniofacial region, cerebral

ventricles, or peritoneal and pericardial cavities. No histologic effects were observed in the 500 mg/kg bw group, and the only cardiovascular alterations during the first 30 min after exposure were two cases of anterior cardinal vein dilation. The study authors hypothesized that cardiovascular alteration may be a mechanism for resorptions and malformations observed after hydroxyurea treatment.

Strengths/Weaknesses: An appropriate number of animals was used. Animals were assigned randomly to experimental groups. The test chemical was defined, but its purity was not stated. There was no reported chemical verification of dosing preparations. The gestational age of animals and duration of exposure were appropriate. Appropriate endpoints were observed, and the endpoints were observed at appropriate life stages. Data were reported in appropriate detail. The attempt at physiology-based teratology with attention to microvascular changes as a possible mechanism of toxicity is a strength. The treatment at a single developmental stage is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process and shows embryonic toxicity of exposure to hydroxyurea in rabbits. The study also provides a proposed mechanism for resorption.

Millicovsky et al. (1981), supported by the American Heart Association, examined the effects of hydroxyurea on hemodynamics in pregnant rabbits. On GD 10 (day of mating = GD 0), catheters were inserted in 10 New Zealand White rabbits. On GD 12, hemodynamics were studied in rabbits s.c. injected with 750 mg/kg bw hydroxyurea **[purity not given]**. Circulatory effects were quantified using radioactive microspheres that were introduced into the left ventricle. Blood pressure was monitored by transducer, and heart rate was assessed by cardiotachometer. Changes in hemodynamics were compared to baseline values. **[No statistical analyses were reported.]** Clinical signs observed immediately after hydroxyurea exposure included dilated pupils, shallow breathing, and hyperventilation. At the same time, alterations in systemic and uterine circulation were observed. Maximum effects of changes compared to baseline values were observed at 1–2 min after hydroxyurea exposure, and the values did not return to baseline by 10 min post-exposure. After hydroxyurea exposure, systolic blood pressure increased by 53%, initial bradycardia was followed by rebound tachycardia, cardiac output decreased by 39%, and total vascular resistance increased by 134%. Hydroxyurea-induced effects on uterine circulation included a 77% reduction in uterine blood flow and a 412% increase in uterine vascular resistance. Based on their findings, the study authors concluded that hydroxyurea significantly altered blood pressure and heart rate and induced uterine vasoconstriction, which may be involved in immediate embryotoxicity.

Strengths/Weaknesses: It is not clear that an appropriate number of animals were used in this presumably pilot study. Animals were not assigned randomly to experimental groups. The test chemical was defined, but its purity was not stated. There was no reported chemical verification of dosing preparations. Appropriate endpoints were observed. Data were reported in appropriate detail, but appropriate statistical evaluation was not reported.

Utility (Adequacy) for CERHR Evaluation Process: Although this study suggests that uterine vasoconstriction makes a physiologic contribution to hydroxyurea-induced embryotoxicity, this study is not useful in the evaluation process.

DeSesso (1981b), supported in part by the Orthopedic Research and Education Foundation and the University of Cincinnati Research Council, examined the effects of hydroxyurea on ultrastructural changes in limb buds of rabbit fetuses. On GD 12 (GD 0 = day of mating), New Zealand White rabbits were s.c. injected with saline (n = 9) or 750 mg/kg bw hydroxyurea **[purity not given]** (n = 18). From 15 min to 32 hr after treatment, 1–3 rabbits/group/time period were killed. Embryonic limb buds were examined for histopathologic effects by light microscopy and for ultrastructural alterations by electron microscopy. The effects of methotrexate were also examined but will not be discussed here. Exposure to hydroxyurea resulted in toxicity to limb bud mesenchyme. Changes were first observed at 30–45 min after treatment and included ribosomal dispersion and swollen endoplasmic reticulum cisternae. Starting at 60 min after exposure, chromatin condensed within nuclei and the granular portion of nucleoli became segregated. At the same time, mesenchymal cells became fragmented, and cellular debris was observed in extracellular spaces and cytoplasm of neighboring mesenchymal cells and macrophages as a result of phagocytosis. Signs of damage, including cytoplasmic vacuoles, were observed in endothelial cells of blood vessels. By 4 hr after treatment, cellular debris was observed throughout the mesenchymal compartment and no mitotic figures could be observed. Mitotic figures began reappearing at 16 hr after exposure. The study author concluded that hydroxyurea disrupted intracellular contents through cytolethality.

Strengths/Weaknesses: This study did not use an appropriate number of animals. The test chemical was defined, but the purity was not stated. There was no reported chemical verification of dosing preparations. The gestational age of animals and duration of exposure were appropriate. Data were reported in appropriate detail. Strengths are the quality of the cytology, the demonstration of cell death as a teratogenic mechanism, and the documentation of recovery of mitosis after treatment. Weaknesses are the single treatment dose and developmental stage.

Utility (Adequacy) for CERHR Evaluation Process: This study provides mechanistic information only and is not otherwise useful in the evaluation process.

DeSesso (1981a), supported by MITRE Corporation, examined the effects of the antioxidant propyl gallate on hydroxyurea-induced developmental toxicity in rabbits. New Zealand White rabbits were assigned randomly to groups on GD 12 (GD 0 = day of mating), and groups of six to eight animals were s.c. injected with hydroxyurea **[purity not given]** or hydroxyurea in combination with propyl gallate at the concentrations listed in Table 59. The compounds were injected simultaneously or mixed in beakers for 15–45 min before injection. Controls received ethanol/water vehicle or 634 mg/kg bw/day propyl gallate. Rabbits were killed on GD 29, and implants were examined. Fetuses were weighed and assessed for

Table 59
Comparison of Developmental Toxicity in Rabbits Treated with Hydroxyurea Alone or in Combination with Propyl Gallate

Dose (mg/kg bw) Hydroxyurea	Co-treatment compared to hydroxyurea alone		
	± Propyl gallate	Resorptions	Malformations
650	362	↓ from 42 to 28%	↔
650	634	↓ from 42 to 19%	↓ from 100 to 88%
650	906	↑ from 42 to 85%	↓ from 100 to 0%
650	634 simultaneous injection	↓ from 42 to 20%	100 vs. 97%
650	634 premixed 15–30 min	↓ from 42 to 16–19%	↓ from 100 to 88–89%
650	634 premixed 45 min	↔	↓ from 100 to 82%
750	721	↔	↓ from 100 to 74%
600	585	↔	↓ from 100 to 80%

From DeSesso (1981a).
↑,↓, ↔, Significant increase, decrease, or no change, respectively, compared to treatment with hydroxyurea alone.

viability and external and skeletal malformations. Statistical methods included χ^2, ANOVA, and Duncan multiple range test. Thin layer chromatography was used to determine if there were chemical reactions between hydroxyurea and propyl gallate and to confirm that fetuses were exposed to hydroxyurea and propyl gallate.

Treatment with the vehicle control and propyl gallate resulted in no adverse developmental toxicity effects, as assessed by malformation and resorption rate. Fetal body weights were increased with propyl gallate treatment, but the authors stated that the fetuses looked healthier than fetuses from the other treatment groups. All doses of hydroxyurea induced fetal malformations affecting face, limbs, trunk, and tail. The most prominent malformations included cleft lip and palate, hemimelia, ectrodactyly, and tail defects. As summarized in Table 59 hydroxyurea-induced malformations were attenuated in some cases by co-treatment with propyl gallate, but the effect was dependent on doses of both compounds. In cases where propyl gallate reduced the total hydroxyurea malformation rate, individual malformation types were also reduced. The severity of malformations was reduced as well, as evaluated by fewer missing digits. Although the group exposed to 650 mg/kg bw hydroxyurea +634 mg/kg bw propyl gallate did not experience a significant reduction in malformations, the spectrum of malformations was reduced. At some doses of propyl gallate, hydroxyurea-induced resorptions were reduced, but higher doses of propyl gallate increased resorption rates and maternal mortality. In the study examining the effects of simultaneous injection with hydroxyurea and propyl gallate compared to mixing solutions for 15–45 min before injection, increased effectiveness in protecting against malformations was observed with premixing (Table 59). Equal protection against resorptions was observed for co-administration and premixing for 15–30 min, but premixing hydroxyurea and propyl gallate for 45 min did not protect against resorptions. Thin layer chromatography showed no reaction or breakdown products of hydroxyurea or propyl gallate and verified that hydroxyurea and propyl gallate were present in fetuses.

In a second study described in this study, rabbits were treated on GD 12 with 650 mg/kg bw hydroxyurea alone or in combination with 634 mg/kg bw propyl gallate. **[A total of 20 rabbits were treated, but the number treated/group was not indicated.]** Rabbits were killed at 2, 4, 8, and 16 hr after exposure, and fetuses were examined histologically. Exposure to hydroxyurea resulted in necrosis, characterized by pyknotic nuclei and basophilic debris within limb-bud mesenchyme, beginning 2 hr after exposure. Co-treatment with propyl gallate apparently delayed necrosis; the effect was not observed up to 4 hr after treatment. Some evidence of necrosis began appearing 8 hr after treatment and increased 16 hr after treatment, but severity was less than that observed 4 hr after exposure to hydroxyurea alone. The study author concluded that the results of this study showed clearly the effectiveness of propyl gallate in ameliorating hydroxyurea-induced embryotoxicity in fetal rabbits.

Strengths/Weaknesses: This study reports a well-designed interaction study that showed that a key toxic event (cell death) can be reduced by co-treatment with propyl gallate. The use of multiple doses and multiple forms of interaction between the two chemicals is a strength. An appropriate number of animals was used. Animals were assigned randomly to experimental groups. The test chemical was defined, but the purity was not stated. There was chemical verification of dosing preparations. The age of animals and duration of exposure were appropriate. Data were reported in appropriate detail, and appropriate statistics were used.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process and suggests that the effects of exposure to hydroxyurea may be mitigated by concurrent exposure to an antioxidant.

DeSesso and Goeringer (1990a), funded by MITRE Corporation, examined the effects of antioxidant compounds on hydroxyurea-induced developmental toxicity in rabbits. On GD 12 (GD 0 = day of copulation), New Zealand White rabbits were assigned randomly to groups of 7–10 animals and s.c. injected with hydroxyurea or antioxidants. Treatments included 650 mg/kg bw hydroxyurea **[purity not given]** in ethanol/water vehicle (positive control) or 950 mg/kg bw ethoxyquin or nordihydroguaiaretic acid (antioxidant controls). A negative control group was left untreated. Additional

groups were s.c. injected with 950 mg/kg bw ethoxyquin or nordihydroguaiaretic acid 15–30 min before s.c. injection with 650 mg/kg bw hydroxyurea. Rabbits were killed on GD 29, and fetuses were counted, weighed, and assessed for viability and gross and skeletal malformations. Data were analyzed on a fetus and litter basis, and because no litter effects were observed, the data were analyzed on a fetus basis. Statistical analyses included χ^2, Fisher exact test, ANOVA, Duncan multiple range test, and Student's *t*-test.

There were no increases in resorptions or fetal malformations in groups treated with ethoxyquin or nordihydroguaiaretic acid. Fetal body weights in the ethoxyquin or nordihydroguaiaretic acid groups were significantly higher than in the unhandled control group. Treatment of pregnant rabbits with hydroxyurea did not affect fetal viability but resulted in a 100% malformation rate and reduced fetal body weight **[by 12%]** compared to the untreated controls. The types of malformations observed with hydroxyurea treatment included cleft lip, cleft palate, and defects of limbs and tails. When ethoxyquin or nordihydroguaiaretic acid were administered before hydroxyurea, the malformation rates were significantly lower (83–87% rate; $P < 0.001$ or 0.01) compared to treatment with hydroxyurea alone. Specific types of malformations (e.g., cleft lip/palate, hemimelia, ectrodactyly, and tail defects) were also reduced with co-exposure to ethoxyquin or nordihydroguaiaretic acid and results attained statistical significance for most malformation types. A decrease in severity of malformations was suggested by significantly greater number of digits with co-exposure to ethoxyquin or nordihydroguaiaretic acid. Compared to treatment with hydroxyurea alone, fetal body weight was significantly increased **[by 8%]** when rabbits received nordihydroguaiaretic acid before hydroxyurea exposure.

In a second study reported in the article, GD 12 rabbit embryos were examined at 4, 8, or 12 hr after maternal treatment with either 650 mg/kg bw hydroxyurea, 950 mg/kg bw ethoxyquin, 950 mg/kg bw nordihydroguaiaretic acid, or hydroxyurea in combination with each antioxidant compound at the same doses listed above. Three to five embryos/litter were examined for necrosis. Treatment with ethoxyquin or nordihydroguaiaretic acid did not affect microscopic anatomy of hindlimb buds. Mesenchymal cell necrosis was observed in limb buds of hydroxyurea-exposed animals beginning at 4 hr after exposure. When rabbits were treated with ethoxyquin or nordihydroguaiaretic acid before hydroxyurea exposure, there was no or little evidence of necrosis at 4 hr, but necrosis was increased at 8 and 12 hr after exposure. The authors noted that although many embryos had no evidence of necrosis at 4 hr after treatment with ethoxyquin+hydroxyurea, necrosis was indistinguishable from that in embryos exposed to hydroxyurea alone in 20% of embryos. Based on the results of these studies, the study authors concluded that the antioxidant properties of ethoxyquin and nordihydroguaiaretic acid interfered with the rapidly occurring toxicity induced by hydroxyurea, which may have resulted in the amelioration of hydroxyurea-induced developmental toxicity in rabbits.

Strengths/Weaknesses: An appropriate number of animals was used. Animals were randomly assigned to experimental groups. The test chemical was defined, but the source and purity were not stated. There was no reported chemical verification of dosing preparations. The age of animals and duration of exposure were appropriate. Data were reported in appropriate detail, and appropriate statistics were used. The mechanistic approach to the role of cell death and the exploration of the role of antioxidants as anti teratogens are strengths.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process as a mechanistic study suggesting that the effects of exposure to hydroxyurea may be mitigated by concurrent exposure to antioxidants.

DeSesso and Goeringer (1990b), supported by MITRE Corporation, conducted a series of studies to examine possible mechanisms of hydroxyurea-induced developmental toxicity in New Zealand White rabbits. The first experiment examined the effects of intrauterine injection. On GD 12 (GD 0 = day of mating), individual implantation sites were injected with either 180 μg hydroxyurea **[purity not given]**, 180 μg hydroxyurea+27 μg propyl gallate, or water/alcohol vehicle. Four hours after injection, embryos were examined for cell death, especially in limb buds. **[The numbers of dams and fetuses treated and examined were not reported.]** In limb buds of embryos exposed to hydroxyurea, basophilic particles of extracellular debris were present and mitotic figures were not observed in mesenchyme or ectoderm. In embryos exposed to hydroxyurea+propyl gallate, limb buds appeared normal, but the numbers of mitotic figures appeared to be reduced compared to controls.

In a second experiment, three to five pregnant rabbits/group were s.c. injected with either 650 mg/kg bw hydroxyurea or 650 mg/kg bw hydroxyurea +634 mg/kg bw propyl gallate. Rabbits were killed between 15 min and 8 hr after treatment, and individual embryos were removed and analyzed for hydroxyurea content using a colorimetric assay. Statistical analyses included Student's *t*-test. In groups treated with hydroxyurea or hydroxyurea+propyl gallate, hydroxyurea levels rose steadily for 3 hr. Concentrations remained steady at ~2.8–3.2 μg hydroxyurea/mg protein from 3–6 hr after treatment and then began declining 8 hr after treatment. The only time point at which a significant difference was observed in hydroxyurea level in the groups treated with and without propyl gallate was at 4 hr; at that time period levels of hydroxyurea were 16% higher in the group that was not exposed to propyl gallate.

In a third experiment, ^{3}H-thymidine incorporation into embryonic DNA was determined for groups of three litters (20–25 embryos/group) that had been exposed on GD 12 to either hydroxyurea, propyl gallate, or vehicle. **[Doses administered and specific method of injection were not reported.]** Rabbits were injected with ^{3}H-thymidine at 1 hr after treatment and killed 1 hr later. Statistical analyses included ANOVA and Duncan multiple range test. Treatment with hydroxyurea alone and hydroxyurea in combination with propyl gallate resulted in a significant ~10-fold reduction in ^{3}H-thymidine incorporation. Based on the findings of the three experiments, the study authors concluded that attenuation of hydroxyurea-induced developmental toxicity, as evidenced by delayed onset of necrosis, resulted from an interaction between hydroxyurea and propyl gallate that

was independent of hydroxyurea uptake by the embryo or DNA synthesis in the embryo.

Strengths/Weaknesses: An appropriate number of animals may or may not have been used. Animals may or may not have been assigned randomly to experimental groups. The test chemical was defined, but the purity was not stated. There was no reported chemical verification of dosing preparations, but embryo content of hydroxyurea was measured. The age of animals and duration of exposure were appropriate. Data were reported in appropriate detail, and appropriate statistics were used. The attempt to assess the mechanism of the hydroxyurea-propyl gallate interaction described previously is a strength. The mechanistic assessment, however, hit a biochemical roadblock.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process in confirming the central role of DNA synthesis inhibition in the biologic effects of hydroxyurea.

DeSesso et al. (1994), supported by MITRE Corporation, examined the effects of *d*-mannitol, a specific scavenger of hydroxyl free radicals, on hydroxyurea-induced developmental toxicity in rabbits in a series of studies. In the first study, six to seven New Zealand White rabbits/group were s.c. injected on GD 12 (GD 0 = day of gestation) with 650 mg/kg bw hydroxyurea **[purity not given]** (positive control group), 550 mg/kg bw *d*-mannitol, or saline. Experimental groups were given 650 mg/kg bw hydroxyurea+550 mg/kg bw *d*-mannitol or 650 mg/kg bw hydroxyurea+550 mg/kg bw xylose. Xylose served as an osmotic control. Rabbits were killed on GD 29, and fetuses and implantation sites were examined. Viable fetuses were weighed and examined for gross and skeletal malformations. Statistical analyses were conducted with the fetus and litter as the experimental unit. Statistical analyses included χ^2 test, Fisher exact test, ANOVA, Duncan multiple range test, Kruskal-Wallis test, or Student's *t*-test. No statistically significant increases in resorptions or malformed fetuses or decreases in fetal weight were observed after exposure to *d*-mannitol. Exposure to hydroxyurea resulted in a 100% malformation rate and decreased fetal weights **[by ~30%]** compared to the saline controls. The malformations observed most frequently were limb-reduction deformities, including reduced numbers of digits. Other malformations included cleft lip and palate and micrognathia. Co-exposure to hydroxyurea and *d*-mannitol resulted in a lower litter malformation rate (93%) and higher fetal body weight (~6% increase) compared to exposure to hydroxyurea alone. Incidences of specific types of malformations (e.g., micrognathia, cleft lip and palate, hemimelia, ectrodactyly) were reduced and number of digits was increased when *d*-mannitol was co-administered with hydroxyurea. No changes in hydroxyurea-induced developmental toxicity were observed with co-exposure to xylose.

In a second experiment, rabbits were s.c. injected on GD 12 with 650 mg/kg bw hydroxyurea, with and without co-exposure to 550 mg/kg bw mannitol or xylose. Three rabbits/group/time interval were killed at 3, 4, 6, or 8 hr after treatment for histologic evaluation of necrosis in embryos. Additional rabbits were treated with 550 mg/kg bw mannitol, 550 mg/kg bw xylose, or saline and embryos were examined at 8 hr after injection. Three or five embryos/litter were examined with particular attention to the limb buds. At 3–8 hr after exposure, hydroxyurea induced cytotoxicity in limb bud mesenchyme that was characterized by pyknotic nuclei and basophilic intercellular debris. When *d*-mannitol was co-administered with hydroxyurea, mesenchymal debris at 4 hr was reduced greatly compared to groups exposed to hydroxyurea alone. By 8 hr after exposure to hydroxyurea and *d*-mannitol, debris in the mesenchyme was increased but cell death did not appear to be quite as extensive as observed at 4 hr after exposure to hydroxyurea alone. Co-exposure to xylose did not attenuate the effects of hydroxyurea in limb buds.

In a third experiment, individual implantation sites were injected on GD 12 with 180 μg *d*-mannitol, 180 μg xylose, or saline before s.c. injection of the maternal rabbit with 650 mg/kg bw hydroxyurea. At 3, 4, 5, or 8 hr after hydroxyurea injection, rabbits were killed and embryos were examined for cell effects in limb buds as described above. A minimum of three embryos/litter were examined in each group. Results were consistent with those observed after maternal exposure. At 4 hr after exposure to hydroxyurea and *d*-mannitol, limb bud architecture was similar to that in groups that were not exposed to hydroxyurea. Cellular debris began accumulating in mesenchyme beginning at 5 hr after exposure to hydroxyurea and *d*-mannitol, and by 8 hr, limb buds were similar in appearance to those of embryos exposed to hydroxyurea+saline or xylose. The study authors concluded that results were consistent with studies reporting antioxidant attenuation of hydroxyurea-induced developmental toxicity and hypothesized that hydroxyl free radicals are the proximate species associated with hydroxyurea-induced cell death in rabbit embryos.

Strengths/Weaknesses: An appropriate number of animals was used. The test chemical was defined, but its purity was not stated. There was no reported chemical verification of dosing preparations. The age of animals and duration of exposure were appropriate. Data were reported in appropriate detail, and appropriate statistics were used. A strength is the further insight into potential cellular mechanisms underlying hydroxyurea embryotoxicity. A weakness is the lack of quantification of mesenchymal cell death to relate levels of cell death to levels of observed limb bud defects.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process in consistently showing cell death as a teratogenic mechanism and the ability of mannitol to reduce the level of hydroxyurea-induced toxicity.

DeSesso et al. (2000), supported by Mitretek Biomedical Research Institute, examined the role of the hydroxyl moiety in developmental toxicity induced by hydroxyurea and other compounds in rabbits. On GD 12 (day of mating = GD 0), New Zealand White rabbits were assigned randomly to groups and exposed to saline or hydroxyurea **[purity not given]** 650 mg/kg bw (8.55 mmol/kg bw) by s.c. injection or 205 μg/embryo (2.66 μmol/embryo) by intracelomic injection. In a subsequent study, rabbits were co-injected with 3.0 mmol propyl gallate. Cell death was examined in embryos 4 or 8 hr after direct or intracelomic injection. After s.c. exposure, three to six embryos from three litters were examined for each time point. After intracelomic

Table 60
Comparative Embryotoxicity in Monkeys and Rats Exposed to Hydroxyurea

Dose (mg/kg bw/day)	Exposure (GD)	No. Embryos	Fetal status
Monkey			
250	18 or 21	2	Normal
250	18–19	1	Normal
500	18	1	Small, but no abnormalities
400	18–19	1	Normal
500	23–25	1	Aborted on GD 28
500	21–22	1	Aborted on GD 34
250	22–25	1	Aborted on GD 36
250	21–23	1	Aborted on GD 32
125	21–24	1	Normal
125	22–24	1	Aborted on GD 37
Rat			
250	9	214	12% resorbed, 83% malformed

From Wilson (1971).
GD, gestational day.

injection, the forelimb buds of 9–12 embryos from three to five litters were examined at each time point. The same study was conducted with equimolar concentrations of additional hydroxylamine compounds, including hydroxylamine hydrochloride, *N*-methylhydroxylamine hydrochloride, acetohydroxamic acid, and hydroxyurethane. Effects were compared to those of corresponding amino analogs, including ammonium hydroxide, methylamine, urea, acetamide, and urethane.

After maternal s.c. or embryonic intracelomic exposure to hydroxyurea, cytotoxicity in limb bud mesenchyme was evidenced by the presence of cytoplasmic basophilia, increased granularity of nucleoplasm, cellular fragmentation, and the lack of mitotic figures. The changes were observed as early as 4 hr after exposure and were present after exposure to any of the hydroxylamine compounds, although the effects were most severe for hydroxyurea. When hydroxyurea was co-administered with propyl gallate by s.c. exposure of dam or intracelomic injection of embryos, there was little-to-no debris in forelimb buds at 4 hr after exposure. No effects in the limb bud were observed after exposure to amino analogs. The study authors concluded that exposure of rabbits to hydroxylamine compounds, but not amino analogs, on GD 12 results in cell death within the forelimb mesenchyme that can be attenuated by co-administration of propyl gallate.

Strengths/Weaknesses: The number of litters used was small. Animals were assigned randomly to experimental groups. The test chemical was defined, but its purity was not stated. There was no reported chemical verification of dosing preparations. The age of animals and duration of exposure were appropriate. Data were reported in appropriate detail. It is not clear that appropriate statistical analyses were used. Strengths are the further confirmation of the potential of propyl gallate to function as an anti teratogen under the conditions of the experiments and affirmation of mesenchymal cell death as a potential teratogenic mechanisms for hydroxyurea-induced limb-bud defects.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process for mechanistic assessment of the association between induced cell death and limb-bud defects.

3.2.5 Monkey. This section includes studies conducted in monkeys and studies comparing hydroxyurea effects in monkeys and rodents.

Wilson (1971), supported by the FDA, NIH, and the Pathological Embryology Research Fund from private sources, compared embryotoxicity in rats and monkeys exposed to hydroxyurea. Very limited details were provided in the publication, which appeared to be a summary of a presentation. Other compounds were examined but will not be discussed here. Doses of hydroxyurea administered, day of treatment, and numbers of exposed embryos are summarized in Table 60. **[No information was provided on strain of monkey or rats, purity of hydroxyurea, administration route, treatment of controls, or numbers of dams treated.]** The study author attempted to administer hydroxyurea at comparable embryonic stages in rats and monkeys. However, determining the embryonic stage in monkeys was difficult because day of fertilization could only be determined within ± 1 day and because pregnancy in monkeys could not be diagnosed before GD 19. Early abortion was diagnosed in monkeys when the uterus ceased growing, as determined by rectal palpitation. Rat dams were killed on GD 20 and fetuses were examined for external and internal malformations. Monkey fetuses were removed by hysterotomy on GD 100 and examined for skeletal malformations. Results in monkeys and rats are summarized in Table 60. The study author concluded that there was little evidence of embryotoxicity in monkeys until the dose reached double that given to rats. **[It is not clear how the author reached his conclusion, because abortions were observed in monkeys at doses lower than or equivalent to those given to rats.]**

Strengths/Weaknesses: The number of animals was not appropriate. Animals may or may not have been assigned randomly to experimental groups. The test chemical was not defined, and its purity was not stated. There was no reported chemical verification of dosing preparations. Use of monkeys is a strength, but the superficial presentation of data with many gaps in reporting is a weakness.

Utility (Adequacy) of CERHR Evaluation Process: This study is of historic interest only and is not useful in the evaluation process.

Wilson et al. (1975), supported by the March of Dimes and FDA, compared embryotoxicity of hydroxyurea in rats and monkeys. Distribution of hydroxyurea was also examined in both species and is discussed in Section 2.2.2. **[In neither rat nor monkey study was there an indication that results were analyzed statistically.]**

Wistar rats were i.p. injected with hydroxyurea **[purity not given]** at 100, 137, or 175 mg/kg bw/day on GD 9–12 (GD 0 = day of vaginal sperm). On GD 20, rat embryos were weighed and examined for viability and skeletal and visceral defects. Results were compared to historical data collected in the laboratory over a 7-year period. The study authors noted an increased malformation rate in

Table 61
Exposure Regimens and Results Observed in Rhesus Monkeys Exposed to Hydroxyurea During Prenatal Development

Exposure regimen	Results
Single dose of 1,000 mg/kg bw on GD 30 or cumulative dose >500 mg/kg bw administered on ≤4 days between GD 21 and 27 ($n = 7$)	Abortion in 6/7 monkeys
Cumulative dose of 500 mg/kg bw on GD 42–45 ($n = 1$)	Abortion
A single or cumulative dose of ≤500 mg/kg bw on GD 18–27 or a cumulative dose of 1,100 mg/kg bw on GD 21–32; hysterotomy was conducted at GD 100 ($n = 8$)	Malformations in ribs and vertebrae in 4 offspring; growth retardation in 2/4 structurally normal offspring; death of 1 fetus before hysterotomy
100 mg/kg bw/day on GD 21–31 ($n = 1$)	Malformations in axial skeleton and cardiovascular system, a single umbilical artery, polysplenia, large supernumerary kidney

From Theisen et al. (1973).
GD, gestational day.

rats exposed to hydroxyurea at 137 and 175 mg/kg bw/day. Respective malformation rates in the historic control versus the 137 and 175 mg/kg bw/day groups were 1.2 versus 40.0, and 55.9%. Ocular and cerebral malformations were observed mainly. **[Although not discussed by study authors, the same doses also appeared to increase incidences of dead or resorbed fetuses. Incidences in the control and each respective group were reported at 5.4, 16.7, and 13.3%.]**

Rhesus monkeys were i.v. injected with 100 mg/kg bw/day hydroxyurea on GD 23–32, 27–36, or 31–40. The day of vaginal sperm detection was considered GD 0; the study authors noted that this method of determining gestational age may have resulted in estimates being inaccurate by 24 hr. At GD 32, 36, and 40, one to nine embryos/time period were weighed and examined for heart-beat and external abnormalities. Values were compared to ~1–2 control fetuses from each approximate age group. **[It did not appear that control monkeys were exposed concurrently.]** All monkey embryos were viable and none had external malformations. The authors stated that internal malformations could not be ruled out because they were not examined in this study. Based on body weights well below the ranges considered appropriate for control animals, the study authors identified growth retardation in two of six 32-day-old embryos and one of four 40-day-old embryos in the hydroxyurea group. It was noted that control values provided only a crude basis of comparison due to the small numbers of animals and variability of data. **[It appears that the control group consisted of one to four animals/age group.]**

The study authors concluded that rats were more sensitive to hydroxyurea developmental toxicity than monkeys, although the difficulty in exposing the two species during the same periods of embryo development at equivalent doses was acknowledged. **[The conclusions appeared to be based on earlier studies (possibly Theisen et al., 1973) that examined internal malformations in monkeys.]**

Strengths/Weaknesses: The number of animals was not appropriate. The test chemical was defined, but its purity was not stated. There was no reported chemical verification of dosing preparations. The age of animals and duration of exposure were appropriate. Data were not reported in appropriate detail, and appropriate statistics were not used. Use of monkeys is a strength but the use of a single dose level is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Theisen et al. (1973), supported by FDA, examined developmental toxicity in rhesus monkeys exposed to hydroxyurea. In the study, hydroxyurea was administered i.v. to 16 pregnant monkeys. **[The authors presented information for 17 monkeys.]** Information from the study appears to be available only as an abstract. Exposure regimen and study results are summarized in Table 61. The study authors concluded that hydroxyurea exposure during the early organogenetic period results in instability of somatic segmentation and effects on visceral development. **[Information from the abstract was summarized because it appeared to be the only source documenting malformations in monkeys. However, abstracts are not considered in conclusions made by the Expert Panel.]**

3.2.6 Hamster. **Ferm (1966)**, supported by the U.S. Public Health Service, examined developmental toxicity in hamsters exposed to hydroxyurea. The effects of urethane were also examined but will not be discussed here. Golden hamsters were i.v. injected with isotonic saline or 50 mg hydroxyurea **[purity not given]** on GD 8 (GD 1 = day after mating). **[Based on body weights reported for hamsters, the hydroxyurea dose was estimated at 400–500 mg/kg bw.]** Groups of three to nine hamsters were killed at 24, 48, and 72 hr (GD 9, 10, 11, or 12) after hydroxyurea exposure. Implantation sites were assessed and embryos were examined for malformations. There were 40–106 embryos available for examination at each time period. **[Time of evaluation was not specified for the 8 litters in the control group. No statistical analyses were conducted. Very limited protocol details were provided.]** Exposure to hydroxyurea increased numbers of resorbed or dead embryos; embryo deaths were reported at 100% by GD 12. **[Resorption rate was not reported for the control group.]** No abnormal embryos were observed in the control group. Percentages of abnormal embryos in the hydroxyurea group were reported at 66% on GD 9, at 75% on GD 10, and at 44% on GD 11. The types of abnormalities observed included exencephaly, spina bifida, cardiac tube defects, and open

neural tube. The study authors concluded that hydroxyurea induces developmental malformations in hamsters. **[These data appeared in part in an earlier letter to the editor (Ferm, 1965).]**

Strengths/Weaknesses: An appropriate number of animals were used. The test chemical was defined, but its purity was not stated. There was no reported chemical verification of dosing preparations. The age of animals and duration of exposure were appropriate. Data were not reported in appropriate detail, and appropriate statistical analyses were not used. Although the presence of clear embryotoxicity is a strength, use of a single high dose level with 100% embryo lethality prevented exploration of a dose-response relationship.

Utility (Adequacy) for CERHR Evaluation Process: This study can be used only to infer that hydroxyurea is teratogenic in the hamster.

3.2.7 In vitro studies in mammalian species

Studies providing the most detailed information on developmental toxicity after exposure of mouse or rat embryos to hydroxyurea are presented before studies focusing on mechanisms of toxicity. The studies are then presented in order of year of publication.

Warner et al. (1983), supported by the EPA, compared hydroxyurea-induced developmental toxicity in in vivo and in vitro mouse assays. On GD 9 (GD 1 = day of vaginal plug), ICR mice were i.p. injected with 300 mg/kg bw hydroxyurea **[number treated not specified, hydroxyurea purity not given]**. At least six dams in the control group were administered the saline vehicle. Mice were killed 48 hr after hydroxyurea treatment, and implantation sites, gross malformations, protein levels, and somite numbers were examined. Examinations were conducted in 72 embryos from the control group and 164 embryos from the hydroxyurea group. Data for endpoints other than malformations were analyzed by Fisher exact test and Tukey-Kramer method. **[It did not appear that malformation data were statistically analyzed.]** Malformations were observed in 45% of treated embryos and 1% of control embryos. The types of abnormalities observed included exencephaly, phocomelia, and incomplete rotation. In the hydroxyurea group, significant reductions were observed for somite numbers **[10% reduction compared to control]** and protein concentrations **[50% reduction]**. Toxicokinetic endpoints were examined and are discussed in Section 2.2.2.

In the in vitro study, ICR mice were killed on GD 9 and 10–17 embryos/group were cultured in hydroxyurea at concentrations of 125 mg/L for 1 hr, 250 mg/L for 1 hr, 300 mg/L for 0.5 hr, or 500 mg/L for 0.5 hr. Embryos were then rinsed and incubated in untreated culture media for the remainder of the 48-hr culture period. Embryos with five or six somites were included in all treatment groups. In addition, a group of embryos with three or four somites was exposed to 300 mg/L hydroxyurea for 0.5 hr. The peak maternal plasma concentration of hydroxyurea was measured at ~300 mg/L in rats dosed with 300 mg/kg bw hydroxyurea, a teratogenic dose. Two groups of 12–17 control embryos, one with three to four somites and another with five to six somites, were exposed to the saline vehicle. Endpoints evaluated at the end of the culture period included heart beat, yolk sac circulation, gross malformations, and numbers of somites. Data for endpoints other than malformations were analyzed by Fisher exact test and Tukey-Kramer method. **[It did not appear that malformation data were analyzed statistically.]**

In a comparison of embryos with three to four and five to six somites exposed to 300 mg/L hydroxyurea for 0.5 hr, decreased yolk sac circulation and increased malformation rate (18 vs. 0% in controls) were the only effects observed in embryos with five to six somites. Effects in embryos with three to four somites included decreased heartbeat, yolk sac circulation, and protein levels as well as an increased malformation rate (41 vs. 7% in controls). The lowest concentration to adversely affect development of embryos with five to six somites was 250 mg/L; at this concentration, all endpoints examined were adversely affected and the malformation rate was 100%. Similar findings were observed at 500 mg/L, with the exception of a lack of significant effect on somite number. Malformations observed in vitro were similar to those observed in vivo and included exencephaly, abnormal limb-bud development, and abnormal rotation at 300 mg/L, craniofacial defects at 250 mg/L, and facial hypoplasia, exencephaly, gastroschisis, and phocomelia at 500 mg/L. The study authors concluded that effects observed in vivo were reproduced in vitro, but that both concentration and duration of exposure are important factors to consider for in vitro studies.

Strengths/Weaknesses: The comparison of in vitro and in vivo effects and the attempt to provide dose-response data are strengths, although the lack of dose response in the in vivo studies is a weakness. The lack of experimental detail and the apparent lack of statistical analysis of malformation data are additional weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study provides dose-response data and validation of in vivo–in vitro comparisons, but its utility is limited by lack of experimental detail.

Hansen et al. (1995), of the FDA, examined the effects of deoxyribonucleotides on hydroxyurea-induced toxicity in rat and mouse embryos in vitro. Embryos were obtained from CD rats on GD 10 and from CD-1 mice on GD 8 (GD 0 = day of vaginal plug). The embryos were incubated in media containing hydroxyurea at 0, 200, 300, or 500 mg/L for 1 hr, rinsed, and incubated in rat serum containing deoxycytidine monophosphate or deoxyadenosine monophosphate at 0, 50, 100, 200, or 400 μM for 43 hr. Embryo growth and development were evaluated in 7–91 rat embryos/group and 6–89 mouse embryos at the end of the culture period. Nucleotide pool levels were measured in 6–10 rat embryos using high performance liquid chromatography. Data were analyzed by ANOVA followed by Duncan test.

In rat embryos, morphologic score and number of somite pairs were reduced significantly with exposure to ≥200 mg/L hydroxyurea, and crown-rump, head length, DNA content, and protein content were reduced at ≥500 mg/L hydroxyurea. Incubation of rat embryos in deoxyadenosine monophosphate without hydroxyurea exposure adversely affected all growth and developmental endpoints at the high dose (400 μM) and head length and DNA content at the mid dose (200 μM). Exposure of embryos to the mid dose (200 μM) of deoxycytidine monophosphate without hydroxyurea exposure improved most growth and developmental endpoints. In a study to evaluate the effects of deoxyribonucleotides on hydroxyurea-induced toxicity,

the hydroxyurea concentration was 300 mg/L and concentrations of the deoxyribonucleotides were 50–400 μM. Exposure to 200 μM deoxyadenosine monophosphate after hydroxyurea exposure increased numbers of somite pairs but adversely affected crown-rump and head length and DNA and protein content compared to hydroxyurea exposure alone. Exposure to 400 μM deoxyadenosine monophosphate after hydroxyurea exposure improved morphologic score compared to exposure to hydroxyurea alone, but the low dose of adenosine monophosphate (50 μM) affected morphologic score adversely. Crown-rump and head length improved after exposure to 50 μM deoxycytidine monophosphate after hydroxyurea exposure compared to exposure to hydroxyurea alone. Hydroxyurea exposure alone at 300 mg/L decreased nucleotide pool levels in embryos, affecting guanine triphosphate most severely. Addition of 50 μM deoxyadenosine monophosphate increased pool levels but most remained lower than controls.

In mouse embryos, morphologic score was reduced at ≥200 mg/L hydroxyurea and number of somite pairs, crown-rump length, head length, DNA content, and protein content were reduced at 300 mg/L hydroxyurea. Effects were more severe in mice than rats. Exposure of mouse embryos to deoxyadenosine monophosphate alone at 25 or 50 μM had no effect on growth or developmental endpoints; these endpoints were affected adversely by exposure to the highest dose (200 μM) of deoxycytidine monophosphate alone. In a study to evaluate the effects of deoxyribonucleotides on hydroxyurea-induced toxicity, hydroxyurea exposure occurred at 300 μg/mL and deoxyribonucleotides concentrations were 25 and 50 μM deoxyadenosine monophosphate and 25, 100, and 200 μM deoxycytidine monophosphate. Exposure of mouse embryos to deoxyadenosine monophosphate after hydroxyurea exposure resulted in no significant change in growth or developmental endpoints compared to exposure to hydroxyurea only. Exposure of mouse embryos to deoxycytidine monophosphate at 25 μM or greater concentrations after hydroxyurea exposure resulted in slightly improved morphologic score, number of somite pairs, and crown-rump and head length compared to hydroxyurea exposure only.

The study authors noted that the mouse embryo is more sensitive to hydroxyurea-induced toxicity than is the rat embryo. They concluded that high doses of deoxycytidine monophosphate and deoxyadenosine monophosphate were toxic to rat and mouse embryos. Deoxyadenosine monophosphate had no consistent effect, and deoxycytidine monophosphate only slightly improved growth and development after hydroxyurea exposure. Therefore, the study authors concluded that developmental toxicity induced by hydroxyurea is not due solely to alterations in deoxynucleotide pool levels.

Strengths/Weaknesses: An appropriate number of animals were used. The test chemical was defined, but its purity was not stated. Data were reported in appropriate detail, and appropriate statistical methods were used. The assessment of species differences and the generation of dose-response data are strengths. This study represents an excellent use of in vitro methodology.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process with results suggesting that mouse embryos are more sensitive to hydroxyurea effects than are rat embryos.

Miller and Runner (1978), supported by NIH, National Institute of Dental Research, and the National Science Foundation, examined the effect of hydroxyurea exposure on thymidine uptake by mouse embryos. Embryos at the 10–12 somite stage were obtained from mice **[strain not reported]** 8.5 days after detection of a vaginal plug. Four embryos/dish were incubated for 30 min in media containing ^{3}H-thymidine. Some embryos were incubated in hydroxyurea 4×10^{-3} M **[304 mg/L]** for 15 min before addition of ^{3}H-thymidine. Embryos were then sectioned and prepared for autoradiography. Different tissues of the embryo were examined using 6–10 embryos obtained from three to six dams/group. Data were analyzed by χ^2 test. Exposure to hydroxyurea significantly reduced the labeling index (incorporation of thymidine into nuclei) in the yolk sac, open midgut endoderm, anterior gut portal, posterior gut portal, amnion, right somatopleure, left somatopleure, and posterior gut tube. Depending on tissue, labeling was inhibited by 12–85%. Mean numbers of grains were above background but clustered in the low end of the control range (i.e., shifted to the left) in the hydroxyurea group. In contrast to controls in which asymmetry in labeling frequency and intensity was observed in the somatopleure, asymmetry was equalized after hydroxyurea exposure. The study authors concluded that selective reduction of thymidine intake and equalizing of asymmetrical proliferation rates may represent mechanisms of hydroxyurea-induced abnormalities of morphogenesis.

Strengths/Weaknesses: The use of in vitro methodology with ^{3}H-thymidine autoradiography is a strength, but the single concentration and single stage of development are weaknesses. This study provides some insight into the differential toxicity of hydroxyurea within tissues, depending on their mitotic potential at the time of exposure; however, the study seems to have been underpowered to show differences in labeling index between tissues.

Utility (Adequacy) of CERHR Evaluation Process: This study has some utility from a mechanistic standpoint.

Coakley et al. (1986), support not indicated, included hydroxyurea as a positive control in a study to measure thymidine uptake in rat embryos after in vitro exposure. Four LAC-P rat embryos/group were obtained on GD 10 (day of vaginal plug = GD 1) and incubated in media containing hydroxyurea at 0 or 2.5 mM **[190 mg/L]**. Embryos were then incubated in media containing thymidine. Data were analyzed by ANOVA. Thymidine uptake was significantly inhibited by hydroxyurea exposure. The authors did not express conclusions relative to hydroxyurea.

Strengths/Weaknesses: Data were not reported in adequate detail. The inhibition of DNA synthesis identified in this in vitro study is consistent with the historical database. The use of one concentration and one stage of development is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study has some utility from a mechanistic standpoint.

Zwierzchowski et al. (1986), supported by the Polish Academy of Sciences, examined hydroxyurea briefly in a

study focusing on the effects of polyamines on DNA synthesis and development of mouse preimplantation embryos. In the portion of the study involving hydroxyurea, ^{3}H-thymidine uptake was measured in 3-day-old CFW mouse embryos (at the 8-cell stage) exposed in vitro to hydroxyurea at concentrations of 0, 1, or 5 mM **[0, 76, or 380 mg/L]**. Data were analyzed by Student's *t*-test. In embryos exposed to hydroxyurea, DNA synthesis (as measured by ^{3}H-thymidine uptake) was significantly reduced to 63% of control levels at the low concentration and to 5% of control levels at the high concentration. The authors did not express conclusions relative to hydroxyurea.

Strengths/Weaknesses: Data were not reported in sufficient detail and the analytic method may have been inadequate. The concentration-response data as reported are inconsistent.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Zucker et al. (1998), of the EPA, used a confocal laser-scanning technique to examine apoptosis in CD-1 mouse embryos exposed in vitro to hydroxyurea. Embryos with three to six somite pairs were obtained on GD 8 (GD 0 = day of vaginal plug) and cultured in media containing hydroxyurea at 0, 250, 500, or 1000 μM **[0, 19, 38, or 76 mg/L]** for 24 hr. The embryos were stained in LysoTraker Red and prepared for examination by confocal microscope. Exposure to hydroxyurea increased the amount of naturally occurring apoptosis in the neural tube and otic pit. After exposure to hydroxyurea, most apoptosis occurred in the neural tube, and more apoptosis was observed in the mid- than in fore- or hindbrain. **[It appears that these changes occurred in embryos exposed to ≥250 μM hydroxyurea.]**

Strengths/Weaknesses: This study bears on the mechanism of hydroxyurea embryo toxicity. The lack of clear concentration-response quantification is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study provides mechanistic support to the historic literature.

3.2.8 Chicken. Murphy and Chaube (1964), support not indicated, examined the effects of hydroxyurea exposure on developmental toxicity in chickens. Hydroxyurea in saline vehicle was injected into the yolk sac of 4-day-old Leghorn chicken embryos. Doses were 0.1–0.2 mg/egg for 28 embryos, 0.4–0.5 mg/egg for 38 embryos, and 0.6–1.0 mg/egg for 70 embryos. Eggs were candled daily, and dead embryos were examined for abnormalities. Surviving embryos were killed at 18 days. **[No information was provided on use of controls, and it did not appear that statistical analyses were conducted.]** In embryos that died before the evaluation period, beak defects were observed in 1/12 low-dose embryos, 8/26 mid-dose embryos, and 35/70 high-dose embryos. Abnormalities were observed in only 2/12 embryos of the mid-dose range that survived to the evaluation period at 18 days, and those abnormalities were classified as decreased body weight, micromelia, and short toe. The estimated LD_{50} was 0.4–0.5 mg/egg. The study authors concluded that hydroxyurea is an active teratogen in chickens.

Strengths/Weaknesses: The clear dose-response data are a strength of this study; however, the lack of assessment of stage specificity, the lack of information on controls, and the lack of statistical analysis are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Bodit et al. (1966), support not indicated, applied 0.05 mL aqueous hydroxyurea solutions to the vascular area of Day 3 chicken embryos. Hydroxyurea 0.25 mg was applied in 41 embryos, 0.5 mg was applied in 82 embryos, 0.75 mg was applied in 69 embryos, and 1 mg was applied in 111 embryos. Embryo mortality was 56% at 0.25 mg, 78% at 0.5 mg, 85% at 0.75 mg, and 93% at 1 mg. The proportion of the survivors that were malformed on Day 13–14 increased in a dose-related manner from 27% at the 0.25 mg dose to 61% at the 0.5 mg dose, 86% at the 0.75 mg dose, and 100% at the 1 mg dose. Malformations included brachymelia and curvature of the lower extremities, cleft beak, micrognathia, and atrophy of the upper eyelid. The authors used similar doses of hydroxylamine (as the chloride) and found embryo lethality but not teratogenicity. They concluded that hydroxylamine was unlikely to be the active teratogenic metabolite of hydroxyurea.

Strengths/Weaknesses: The dose-response information is a strength. The lack of controls and stage-specific evaluation are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Iwama et al. (1983), support not indicated, examined potential mechanisms of hydroxyurea-induced malformations in chicken embryos. On Day 4 of incubation, White Leghorn chicken eggs were injected with 800 μg hydroxyurea. Control eggs were injected with the distilled water vehicle. Incubation was continued to incubation day 5, 6, 7, 8, or 9. At the end of the incubation period, leg buds or hindlimbs were removed for measurement of uronic acid level by the carbazole method, DNA concentration by Burton method, and calcium content in bone ash by absorption spectrophotometry. Hindlimbs were examined by light microscopy and then incubated for 2 hr in media containing ^{35}S-sulfate or ^{3}H-glucosamine hydrochloride. Radioactivity levels were then measured in proteoglycan and glycosaminoglycan. **[Statistical analyses were conducted but the methods used were not described.]**

Embryo survival rate was 65% in the hydroxyurea-exposed group. Effects observed with hydroxyurea exposure included reduced body size and shortened, thinned, and bent hindlimbs. Reductions were also observed in limb length on Days 6–9, DNA content on Days 6–8, uronic acid levels on Days 6–8, and calcium content on Days 8 and 9 in this group. Examination of the mesenchymal core from hindlimbs of Day-5 chick embryos exposed to hydroxyurea showed pyknotic nuclei, basophilic debris, large intercellular spaces, and reduced numbers of mitotic figures. At later stages, metachromasia and normal hypertrophy of chondrocytes were observed in the midportion of the femur shaft in hydroxyurea-exposed embryos. Calcification of periosteal bone collar was disrupted on Day 9 in the hydroxyurea-exposed group. Incorporation of ^{35}S-sulfate and ^{3}H-glucosamine into glycosaminoglycan was reduced in the hydroxyurea group on Days 7 and 9, the time of repair or recovery in embryos. The amount of cartilage-specific proteoglycan, an index of chondrogenesis, was reduced in the hydroxyurea-exposed group on

Day 7, as was the ratio of proteoglycan to noncartilagenous proteoglycan. Based on their findings, the study authors concluded that limb defects in chick embryos exposed to hydroxyurea may have resulted from inhibited chondrogenesis and osteogenesis during the repair process.

Strengths/Weaknesses: A strength is the attention to the putative causes of limb defects, including cell death, decreases in mitosis, and the level and extent of chondrogenesis. The lack of dose response is a weakness.

Utility (Adequacy) for CERHR process: This study has utility in considering mechanisms of hydroxyurea toxicity.

3.2.9 Aquatic/amphibian species. Studies are presented below according to species and year of publication.

Baumann and Sander (1984), supported by the German Research Council, examined the effects of hydroxyurea exposure on developmental toxicity in zebrafish. Other compounds were examined but the effects will not be discussed here. Eggs were apparently incubated for 45 min in 10 g/L hydroxyurea dissolved in aquarium water, apparently beginning at Stage 11. **[Exposure conditions were not clearly defined for hydroxyurea.]** Embryo morphology was observed by light microscopy or by time-lapse film. Some embryos were sectioned. Exposure to hydroxyurea delayed proliferation to the point that cells migrated individually rather than as a mass at the start of epiboly. When proliferation and movement of deep cells was inhibited to a greater extent than cell differentiation, organogenesis was initiated before deep cells reached their final destination in the uniform germ shield, resulting in a 20.7% rate of bipartite axiation in the hydroxyurea-exposed group. In hydroxyurea-induced bipartite embryos, tail bud structures formed on both the dorsal and ventral sides, and the neural strand, when visible, was also bipartite. Long portions of notochord were often observed to be completely isolated from other mesodermal organs, and somite development was often abnormal. The authors concluded that a delay in epibolic convergence, which they attributed to reduced cell proliferation, led to a bipartite embryo.

Strengths/Weaknesses: The use of the zebrafish model is a strength, but the study includes no substantive quantitative data.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Murphy and Chaube (1964), support not indicated, examined the effects of hydroxyurea exposure on developmental toxicity in sand dollar and sea urchin embryos. Fertilized sea urchin embryos were exposed to hydroxyurea at 2–50 mg/L sea water. Development of embryos was blocked at the morula stage after exposure to hydroxyurea. Embryos affected by hydroxyurea exposure contained fewer cells that were varied in size and had irregular shapes, abnormally large nuclei, and fine chromosome networks with conspicuous metaphase chromosomes. Arrested development could not be reversed by exposure to purines or pyrimidines. Sea urchin embryos were used to examine chromosomal abnormalities induced by hydroxyurea. Observations in sea urchins treated with unspecified concentrations of hydroxyurea included enlarged nuclei, elongated metaphase chromosomes, anaphase bridging, polyploidy, fragmented chromosomes, and C-mitoses. Thymidine uptake and normal embryo development occurred until the 8-cell stage in sea urchins but were inhibited at later stages. **[Hydroxyurea concentrations that induced effects in sand dollars and sea urchins were not reported clearly.]**

Strengths/Weaknesses: The use of echinoderms in screening for embryotoxicity is interesting, but this study seems to be qualitative. There are no useful quantitative data.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Brachet (1967), support not indicated, examined the effects of hydroxyurea on sea urchin and amphibian eggs. Effects of hydroxyurea were also studied in the alga *Acetabularia mediterranea*, but will not be discussed here. The study was only presented briefly with little information. Nine experiments were conducted with sea urchin eggs (*Paracentrotus lividus* and *Arbacia lixula*) using hydroxyurea concentrations of 10^{-4}–10^{-2} M **[7.6–760 mg/L]**. At all hydroxyurea concentrations, cleavage of sea urchin eggs was blocked at the 4–8-cell stage. Observations in these cells included arrest of nuclei in interphase, swelling, a course network of chromatin, and fibrillar nucleoli lacking RNA. A positive Schiff reaction occurred in the cortex of eggs treated with hydroxyurea 10^{-2} M **[760 mg/L]**. Blocked cleavage was irreversible after $\geq$4-hr exposure to hydroxyurea 10^{-3} M **[76 mg/L]**. Co-exposure to hydroxyurea 10^{-4} M **[7.6 mg/L]** and thymidine 10^{-3} M increased percentages of swimming blastulae, but thymidine did not improve developmental outcome at higher hydroxyurea doses.

Ten experiments on amphibian eggs (*Pleurodeles*, *Xenopus*, *Ambystoma mexicanum*, and *Rana temporaria*) were conducted. Hydroxyurea concentrations of 10^{-3}–10^{-2} M **[76–760 mg/L]** blocked development at the late blastula stage. Observations in these embryos included swollen nucleoli lacking RNA, polycentric mitoses, fragmented chromosomes, and positive Schiff reaction in the cortical layer. Exposure to hydroxyurea 10^{-3} M **[76 mg/L]** at the gastrula stage resulted in various abnormalities including exogastrulation, microcephaly, missing eyes, fusion of somites, and complete or partial absence of the nervous system.

Strengths/Weaknesses: This study presents potentially useful model organisms for testing or mechanistic studies.

Utility (Adequacy) of CERHR Evaluation Process: These models could be useful if combined with newer techniques, but this study by itself is not useful in the evaluation process.

3.2.10 Insects. Oland and Tolbert (1988), supported by NIH, examined the effect of hydroxyurea on developing olfactory glomeruli in the moth (*Manduca sexta*). The focus of the study was to differentiate between the role of glial cells and afferent axons in development of olfactory glomeruli. Thirty-five moths were injected with 9500 mg/kg bw hydroxyurea in distilled water at late Stage 4/early Stage 5 of metamorphosis, the time when neuropilar glial cells are proliferating and neurons have undergone final mitosis. **[A control group was included, but treatment of those animals was not described.]** Most moths were killed at Stage 12 or 13 because Stage 12 is when the antennal lobe becomes histologically mature. A few moths were allowed to develop to Stage 15/16. Brains were examined

by light and electron microscopy. Glial cells were counted in the antennal lobe, and axons were counted in the antennal nerve of one Control and five Stage 12/13 animals of the hydroxyurea group. Electroantennogram recordings were obtained in antennae stimulated with an odorant in two Stage 14–16 moths/sex/group. **[No statistical analyses were reported.]**

Exposure to hydroxyurea resulted in stunted development of antennae in 6% of moths, no development beyond Stage 4/5 in 28% of moths, and death in 23% of moths. In the remaining moths, development was essentially normal until Stage 15/16, at which time development ceased and moths failed to eclose. Abnormalities observed in the hydroxyurea-treated moths were lack of scales and distorted spines on legs, sparse numbers of scales on head and wing, and softened cuticular structures. A variable reduction in numbers of glial cells was observed in Stage 12 moths exposed to hydroxyurea. When glial cell numbers were reduced to ~25% the normal number, glomeruli were not observed in the neuropil. Gross morphology of antennae was not affected by hydroxyurea exposure, although the cuticle was sometimes softer. In the hydroxyurea group, there was no effect on numbers of axons in the antennal nerve in Stage 12/13 moths or response of antennae to odorants in Stage 14–16 moths. The study authors concluded that glial cells are necessary for the formation of olfactory glomeruli in moths.

Strengths/Weaknesses: This study was a sophisticated evaluation of hydroxyurea effects on moth neural development; however, the dose required to produce an effect was very large.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Koundakjian et al. (1996), supported by the Department of Energy and Johns Hopkins University, examined the effects of hydroxyurea exposure on expression of heat shock proteins in genetically engineered *Drosophila melanogaster* embryonic cell cultures containing a β-galactosidase reporter. The main focus of the study was the effect of magnetic fields. Other chemicals were also tested; results with compounds other than hydroxyurea will not be discussed here. The cultures were exposed to magnetic fields of 0, 10, or 100 μT for 16 hr with and without the addition of aqueous hydroxyurea solution at concentrations of 0, 5×10^{-3}, and 5×10^{-2} M **[0, 380, and 3803 mg/L]**. Expression of heat shock proteins hsp23 and hsp70 were measured using an immunoblot technique. Cultures were exposed in triplicate and the experiments were replicated three times. Data were analyzed using ANOVA and Tukey-Kramer multiple comparison test. Exposure to magnetic fields for 16 hr had no effect on expression of heat shock proteins. Hydroxyurea increased hsp23 expression significantly with and without exposure to magnetic fields at 10 μT. In the study examining the effect of magnetic fields at 100 μT, hydroxyurea had no significant effect on hsp23 expression, either alone or in combination with magnetic field exposure. No significant effect was observed for hsp70 expression after exposure to hydroxyurea alone or in combination with magnetic fields. The study authors concluded that exposure to hydroxyurea did not alter the effects of magnetic fields on expression of heat shock proteins.

Strengths/Weaknesses: Data were reported in adequate detail, and appropriate statistical analyses were used.

Utility (Adequacy) for CERHR Evaluation Process: This study is possibly useful in the evaluation process, with results showing that treatment with hydroxyurea increased hsp23 expression in *Drosophila melanogaster* embryonic cells.

3.2.11 Information from drug labels. Drug labels for hydroxyurea describe various developmental toxicity studies that are not known to be publicly available. Teratogenicity associated with hydroxyurea exposure was reported in studies of mice, hamsters, cats, miniature swine, dogs, and monkeys at doses comparable to human doses on a mg/m^2 basis (Bristol-Myers-Squibb, 2001b, 2002, 2004, 2005a,b). Embryotoxicity (e.g., reduced fetal viability, decreased live litter size, and developmental delays) and malformations (e.g., partial ossification of cranial bones, absent eye sockets, hydrocephalus, bipartite sternebrae, and missing lumbar vertebrae) were observed at doses of 180 mg/kg bw/day in rats (~0.8 times the maximum recommended human dose on a mg/m^2 basis) and 30 mg/kg bw/day in rabbits (~0.3 times the maximum recommended human dose on a mg/m^2 basis) (Bristol-Myers-Squibb, 1999, 2001a,b, 2002, 2004, 2005a,b). Growth inhibition and compromised learning ability were observed in rats exposed to ≥375 mg/kg bw hydroxyurea (~1.7 times the maximum recommended human dose on a mg/m^2 basis).

3.2.12 Alternate methods. CERHR identified a number of publications in which hydroxyurea was used in the evaluation of alternate methods for screening compounds for developmental toxicity. These studies use hydroxyurea as a "positive"; that is, a compound that the proposed test is expected to identify as active. Because the studies do not add to the knowledge of potential developmental toxicity induced by hydroxyurea, they will not be summarized here. A list of references for those studies is provided for readers interested in this issue. Those references include: Bantle et al. (1994), Bigot et al. (1999), Bournias-Vardiabasis (1990), Bremer et al. (1999), Courchesne and Bantle (1985), Daston et al. (1995), Dawson and Bantle (1987), Dawson and Wilke (1991), Dawson and Wilke (1992), Finch et al. (1995), Guntakatta et al. (1984), Kavlock et al. (1987), Kemppainen et al. (1996), Khera and Whalen (1988), Kosazuma et al. (1994), Laschinski et al. (1991), Lin (1987), Lynch et al. (1991), Lyng (1989), Nito et al. (1991), Sabourin et al. (1985), Scholz et al. (1999), Schuler et al. (1985), Shiota et al. (1990), Shirazi and Dawson (1991), Spielmann et al. (1997), Steele et al. (1988), Stringer and Blankemeyer (1995), Walmod et al. (2004), and Wickramaratne (1987).

3.3 Utility of Developmental Toxicity Data

3.3.1 Human. There are case reports and case series describing outcome after hydroxyurea exposure in 58 pregnancies. These reports are limited by their uncontrolled nature, by the possible effects of maternal illness and other medications, and by lack of long-term follow-up. There are >30 studies on the use of hydroxyurea in children. The collective utility of these studies was decreased by the use of different hematologic toxicity criteria for withholding

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Table 62
Toxicity in Children Treated with Hydroxyurea for Sickle Cell Disease

				Laboratory values compared to baseline							
							Counts (% decrease)				
n	Dose (mg/kg bw/day)	Duration (months)[a]	Non-hematologic toxicity	Hemoglobin, % increase	Mean corpuscular volume (% increase)	Hemoglobin F (fold increase)	Reticulocyte	Platelet	Leukocyte	Neutrophil	Reference
22	20–25	6	None reported	None	12	3.3	31	None	29	—	Ferster et al. (1996)
93	20–25	12	None reported	7	13	2.3	—	—	—	35	Ferster et al. (2001)
14	20–35	—	Nausea, hair loss	18	24	4.6	30	20	31	31[b]	Jayabose et al. (1996)
13	10–34.7	6–39	None reported	16	18	2.2	None	—	—	—	Scott et al. (1996)
35	20–34	12	Hair loss, nail changes	7	—	—	41	None	—	42	de Montalembert et al. (1997)
29	20–40	12–36	None reported	6	20	4.3	45	13[b]	—	42	Maier-Redelsperger et al. (1998)
17	6.7–32	18.5	Rash, nausea, conjunctivitis, hair loss	15	20	2.2	39	29	—	34	Olivieri and Vichinsky (1998)
19	16.4–31.2	20–66	None reported	17	29	6.7	—	28	46	56	Koren et al. (1999)
84	15–30	6	Pain, nausea, vomiting, infection, headache, diarrhea, rash, bleeding	13	16	2	42	20	32	37	Kinney et al. (1999)
21	20	24	None reported	None	10	None	—	None	20	—	Wang et al. (2001)
122	15–35	6–101	Skin and nail changes, gastrointestinal irritation	18	25	2.5	48	22	44	44	Zimmerman et al. (2004)
8	15–30	12–67	None reported	26	—	2.9	—	None	None	38	Hoppe et al. (2000)
6	15–30	12	None reported	None	33	5.5	None	None	31	44	Miller et al. (2001)
36	8–35	12	None reported	11	19	2	39	36	30	—	Al-Jam'a and Al-Dabbous (2002)
7	15–25	15	Leg ulcer	None	11b	2.2	None	None	—	None	Braga et al. (2005)

[a]Duration of therapy at the time of laboratory data collection.
[b]Change indicated by the study authors although not statistically significant.

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hydroxyurea, the inclusion of multiple sickle cell anemia genotypes, the use of different hydroxyurea maximum doses ranging from ~20–40 mg/kg bw/day (with lower dose used for younger children in the growth study), and different durations of therapy before outcome measures were analyzed. There were few studies with large sample sizes, and many studies included patients from previous studies. Only one study assessed patient compliance with direct objective measures; in other studies, only indirect measures were used. There are no useful data on childhood development after the use of hydroxyurea for malignancy.

3.3.2 Experimental animal. A variety of dosing paradigms were used in experimental animal studies, including different periods, durations, and routes of exposure. The oral and i.p. routes were deemed to be equally relevant due to similar pharmacokinetics. Several experimental animal studies used hydroxyurea as a positive control, providing limited information on developmental toxicity due to the single high dose levels used in these studies. There are several studies in rats, mice, and other species in which more than one hydroxyurea dose level was used, permitting consideration of dose-response relationships. Most of these studies involved administration on a single day of gestation or on a restricted number of days (e.g., GD 9–12), limiting their utility for an evaluation of the entire gestational period. There are two studies (Vorhees et al., 1983a,b) involving postnatal administration of hydroxyurea to developing experimental animals. There are experimental animal studies addressing mechanism of action.

3.4 Summary of Developmental Toxicity Data

3.4.1 Human. Hydroxyurea therapy during pregnancy was described in 32 pregnancies by Thauvin-Robinet et al. (2001). Case reports of an additional 26 pregnancies with hydroxyurea exposure have been published (Table 17). Several cases involved use throughout pregnancy. Hydroxyurea therapy of the pregnant women in these cases was prescribed for the treatment of serious illnesses including hematologic malignancies, essential thrombocythemia, and sickle cell disease. Adverse outcomes reported in some of these cases may have been due to the underlying illness being treated or to concomitant medications rather than to hydroxyurea. For example, common complications of sickle cell disease in pregnancy include low birth weight, prematurity, spontaneous abortion, and pre-eclampsia. There are no data on the long-term effects on offspring born to women exposed to hydroxyurea. There are insufficient data on offspring outcomes after the use of hydroxyurea at different periods or for different durations during pregnancy. Although minor malformations have been observed in the case series, a syndrome of congenital malformations attributable to hydroxyurea was not evident in the case reports taken as a whole.

Reports on the use of hydroxyurea in children have almost exclusively involved therapy of hemoglobinopathies, usually sickle cell disease. Most of the studies showed consistently the same hematologic/myelotoxicity associated with hydroxyurea therapy, summarized in Table 62. Thrombocytopenia and neutropenia have been reported most commonly. The hematologic toxicity was reversible on decreasing or stopping hydroxyurea therapy. The increases in hemoglobin and hemoglobin F and the decrease in reticulocyte counts are noted in Table 62 for completeness but are considered reflections of hydroxyurea efficacy rather than toxicity. Decreases in clotting Factor VIII described in one study (Koç et al., 2003) has also been considered beneficial in decreasing the hypercoagulability of sickle cell disease.

Besides hematologic/myelotoxicity, little-to-no other major toxicity has been noted with hydroxyurea therapy, but there are insufficient long-term data. Adverse effects of therapy have included hair loss and nail and skin hyperpigmentation. Leg ulcers have been noted in children treated with hydroxyurea for sickle cell disease, but leg ulcers may also occur in children with untreated sickle cell disease.

Studies of growth (height and weight) and development in children receiving hydroxyurea are summarized in Table 63. These studies varied in the detail with which evaluation of growth and development was described. In some cases, growth was evaluated by averaging serial heights and weights for children of different ages, a method that is unlikely to be sensitive enough for the purposes of this evaluation. None of the studies identified abnormalities in growth or delays in development, including pubertal progression. Children in these studies ranged from 5–15 years old. There are no studies of reproductive function in people treated with hydroxyurea during childhood or adolescence. There are no data on the effects on subsequent generations after the exposure of developing germ cells to hydroxyurea in utero, during childhood, or during adolescence.

The potential mutagenicity of hydroxyurea in children being treated for sickle cell disease was assessed in two studies. One study reported that 17 children on hydroxyurea for a median of 30 months did not have a statistically significant increase in *HPRT* mutation frequency but had an increase in Vγ-Jβ translocation events compared to children not on hydroxyurea (Hanft et al., 2000). The other study found no increase in Vγ-Jβ translocation events in 34 children treated with hydroxyurea for at least 5 years when comparisons were made with pretreatment values (Zimmerman et al., 2004).

One study reported that serum magnesium was reduced by hydroxyurea in five girls with sickle cell disease (Altura et al., 2002). The study authors proposed that the hypomagnesemia of sickle cell disease may be worsened by hydroxyurea therapy.

There are no studies on the long-term health effects, including carcinogenicity, after childhood exposure to hydroxyurea.

3.4.2 Experimental animal. Studies in pregnant rats using multiple hydroxyurea dose levels are summarized in Table 64. The lowest NOAEL in the rat studies was 50 mg/kg bw/day i.p. on GD 9–12 and the lowest LOAEL was 100 mg/kg bw/day i.p. on GD 9–12, with endpoints of increased external malformation, decreased free-fall reflex, and increased rearing in female offspring (Asano et al., 1983). The lowest BMD_{10} for these endpoints was for external malformation at 74 mg/kg bw/day with a $BMDL_{10}$ of 60 mg/kg bw/day. The lowest BMD_{10} in the data set is 17 mg/kg bw/day, with a $BMDL_{10}$ of 7 mg/kg bw/day for abnormal thoracic vertebral centra after hydroxyurea treatment with 300 or 500 mg/kg bw/day i.p. on GD 11 (Chahoud et al.,

Table 63
Summary of Studies on Growth and Development of Children Treated with Hydroxyurea

Age (yr)	*n*	Dose (mg/kg bw/day)	Findings	Study
10–17	8	14.1–34.7	Children treated for at least 2 years maintained their height and weight percentiles.	Scott et al. (1996)
5.3–18.4	15	15–35	Growth velocities were normal. **[Data were not shown.]**	Rogers (1997)
3–20	35	mean 33–34	Growth velocity assessed by *z*-score was normal after 1 and 2 years of therapy. Sexual maturation was normal **[methods and results not shown for sexual maturation]**.	de Montalembert et al. (1997)
5–15	35–78	15–30	Growth velocity was ≥5th percentile in all children after 6 months ($n = 78$), 1 year ($n = 76$), and 2 years ($n = 35$) of therapy.	Kinney et al. (1999)
0.5–2.3	21	20	Neurodevelopmental assessments did not change over the course of 2 years of therapy. Growth velocity was normal and not different from historical controls. Head circumference percentile was stable.	Wang et al. (2001)
2.5–4.3	11–17	20–30	Growth was normal using standardized curves. Boys increased their weight and height percentiles. Height and weight were higher than historical database of untreated children with sickle cell disease.	Hankins et al. (2005)
5–16	68	30	Height and weight were higher than historical database of untreated children with sickle cell disease. Pubertal transitions occurred at ages comparable to those reported in the historical comparison group.	Wang et al. (2002)
0.5–19.7	233	15–30	Height and weight showed no adverse effect of therapy when averaged across all ages.	Zimmerman et al. (2004)
2–5	8	15–30	There were no deviations in individual growth percentiles, and developmental milestones were attained at appropriate ages.	Hoppe et al. (2000)

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2002). Benchmark doses in both studies were based on two dose levels and on administration of hydroxyurea for limited periods of gestation. In a study with a more traditional regulatory-compliant design (Aliverti et al., 1980) using four hydroxyurea dose levels plus a control and using treatment on GD 6–15, the most sensitive endpoints were postimplantation loss and reduced fetal body weight with NOAEL = 150 mg/kg bw/day and LOAEL = 300 mg/kg bw/day. The lowest BMD_{10} in this study was 125 mg/kg bw/day ($BMDL_{10}$ 114 mg/kg bw/day) for postimplantation loss.

Studies in pregnant mice, all but one of which used multiple hydroxyurea dose levels, are summarized in Table 65. Studies using single dose levels in pregnant rats are summarized in Table 66. Studies in other species are summarized in Table 67. The single dose-level rat studies are consistent with the multiple dose-level studies, because doses of ≥200 mg/kg bw/day given during gestation produced developmental toxicity.

Consistent findings in single and multiple dose-level studies in which hydroxyurea was given to pregnant rats include decreases in fetal growth and viability and dose-related increases in congenital malformations. The malformations reported most commonly in rats are neural tube defects, hydrocephalus, an/microphthalmia, cleft palate, micrognathia, a/ectrodactyly, diaphragmatic hernia, and vertebral abnormalities (Murphy and Chaube, 1964; Scott et al., 1971; Chaube and Murphy, 1973; Brunner et al., 1978; Barr and Beaudoin, 1981; Sugrue and Desesso, 1982; Asano et al., 1983; Price et al., 1985a,b; Asano and Okaniwa, 1987). In mice, malformations included neural tube defect, microcephaly, cleft palate, oligo/poly/syndactyly, hemi/amelia, skull defect, and vertebral abnormality (Roll and Bär, 1969; Kwasigroch and Skalko, 1985; Platzek and Schwabe, 1999; Woo et al., 2004; Yan and Hales, 2005). In rabbits, malformations commonly reported after hydroxyurea included cleft lip/palate, micrognathia, hydrocephalus, a/ectrodactyly, and tail defects (DeSesso and Jordan, 1977; DeSesso and Goeringer, 1990a; Desesso et al., 1994).

There are two studies in which rat pups treated on PND 2–10 showed developmental toxicity with a hydroxyurea dose of 50 mg/kg bw/day (Vorhees et al., 1983a,b). Results included alterations in post-weaning behavior as well as delayed vaginal opening in females, although the results of the two studies were not consistent. Some aspects of postnatal development of the central nervous system in the rat correspond to the prenatal period in humans. Because the dose was administered directly to the offspring, it cannot be compared directly to maternal administration during pregnancy. No other studies using immature experimental animals were located.

Mechanistic studies in rats and mice have suggested that hydroxyurea produces developmental toxicity through inhibition of DNA synthesis with consequent arrest of the cell cycle and cell death (Rajewsky et al., 1971; Chaube and Murphy, 1973; Krowke and Bochert, 1975; Herken, 1984; Platzek and Schwabe, 1999). Rabbit studies have confirmed cell death as a mediator of limb defect, but have suggested a role for cardiovascular effects and oxygen radicals as causes of cell death. The oxygen radical theory has been based on the partial effectiveness of antioxidants in preventing hydroxyurea-induced limb defects (DeSesso, 1981a,b; DeSesso and Goeringer, 1990a,b; DeSesso et al., 2000).

Table 64
Summary of Developmental Toxicity in Multiple-Dose Rat Studies

Strain, route	Endpoint	Dose (mg/kg bw or mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
Sprague-Dawley, "oral" GD 6–15	↑Postimplantation loss	150	300	125	114	—	—	Aliverti et al. (1980)
	↓Mean fetal weight	150	300	164	119	146	101	
	↑Malformations (per fetus)							
	External	150	300	329	293	—	—	
	Visceral	150	300	282	248	—	—	
	Skeletal	150	300	287	243	—	—	
Wistar, i.p. GD 9, 10, 11, or 12[a]	"Teratogenicity"	250	500	—	—	—	—	Murphy and Chaube (1964)
Wistar, i.p. GD 9, 10, 11, or 12[a]	↑Fetal mortality, GD 9	250	375	—	—	—	—	Chaube and Murphy (1966)
	↑Malformations, GD 9	–	≤185	—	—	—	—	
	↑Fetal mortality, GD 10	250	375	—	—	—	—	
	↑Malformations, GD 10	–	≤250	—	—	—	—	
	↑Fetal mortality, GD 11	≥1,000	—	—	—	—	—	
	↑Malformations, GD 11	—	≤375	—	—	—	—	
	↑Fetal mortality, GD 12	750	1,000	—	—	—	—	
	↑Malformations, GD 12	—	≤1,000	—	—	—	—	
Wistar, i.p. GD 12[a]	↑Dead/resorbed implants	250	500	637	565	—	—	Scott et al. (1971)
	↑Malformed survivors	250	500	365	333	—	—	
Sprague-Dawley, i.p. GD 9–12	↓Fetal body weight	100	200	203	187	201	168	Asano and Okaniwa (1987)
	↑Malformations (per male fetus)	100	200	131	133	—	—	
	↑Malformations (per female fetus)	100	200	134	126	—	—	
	↓Male birth weight	100	200	126	154	244	141	
	↓Female birth weight	100	200	201	131	198	123	
	↓Viability index PND 4	100	200	—	—	—	—	
	↓Male body weight PND 21	100	200	227	155	228	150	
	↓Female body weight PND 21	100	200	207	137	205	129	
	↑Malformed PND 21 males	100	200	128	107	—	—	
	↑Malformed PND 21 females	100	200	133	111	—	—	
Wistar, i.p. GD 9–12	↓Fetal body weight	100	200	120	81	79	42	Asano and Okaniwa (1987)
	↑Malformations (per male fetus)	100	200	156	97	—	—	
	↑Malformations (per female fetus)	100	200	153	90	—	—	
Wistar, i.p. GD 11	Fetal body weight	300	500	363	293	328	244	Chahoud et al. (2002)
	Resorptions/litter	300	500	166	93	342	272	
	Litters with:							
	Cleft palate		300	500	454	320	—	—
	Absent forelimb digit 5		300	500	434	312	—	—
	Malpositioned hindlimb		300	500	412	308	—	—
	Abnormal thoracic centra		–	≤300	17	7	—	—
Wistar, i.p. GD12	Hindlimb splay, PND 30–40	—	375	219	168	—	—	Butcher et al. (1973)
	Kinked tail, PND 30–49	375	500	394	355	—	—	
	↓Male weight, PND 50	375	500	Insufficient data for modeling				
	↓Female weight, PND 50	375	500	Insufficient data for modeling				
Hooded, i.p. GD 14	↓Body and brain weight, brain DNA content	—	≤1000	Inadequate demonstration of dose-response relationship				Adlard and Dobbing (1975)
	↑Neonatal mortality at 48 hours	—	≤1000	Inadequate demonstration of dose-response relationship				
Wistar, i.p. GD 9–12	↑External malformations, PND 4	50	100	74	60	—	—	Asano et al. (1983)
	↑External malformations, PND 21	50	100	86	75	—	—	
	↓Free fall reflex in males	50	100	Insufficient data for modeling				
	↑Rearing in females, 4 weeks	50	100	Insufficient data for modeling				
Wistar, i.p. GD 9–12	↑Stillbirth	100	200	135	119	—	—	Asano et al. (1983)
	↓Birth weight, males	100	200	271	146	231	120	
	↑External malformations							
	At birth	100	200	188	158	—	—	
	PND 4	100	200	116	92	—	—	
	PND 14	100	200	110	79	—	—	

Table 64
Continued

Strain, route	Endpoint	Dose (mg/kg bw or mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
	PND 21	100	200	174	110	—	—	
	PND 56	100	200	109	84	—	—	
	↓Viability index	100	200	284	138	—	—	
	↓Free-fall reflex, males, PND 21	100	200	101	72	—	—	

[a]The report provided few details and no statistical analyses. The Expert Panel found this report to be of limited utility.
GD, gestational day; i.p., intraperitoneal; PND, postnatal day.

Expert Panel Conclusions

Evidence is insufficient to conclude that hydroxyurea produces developmental toxicity with exposure during human pregnancy or lactation.

Evidence is sufficient to conclude that hydroxyurea treatment of children age 5–15 years does not produce growth delay at therapeutic doses. There were various durations of follow-up ranging from 6 months to 12 years.

There is insufficient evidence to conclude that hydroxyurea does not affect growth in children under the age of 5 years. There is insufficient evidence to evaluate the effects of hydroxyurea on pubertal progression.

There is no evidence with which to evaluate reproductive function in people treated with hydroxyurea during childhood or adolescence. There are no data on the effects on subsequent generations after the exposure of developing germ cells to hydroxyurea in utero or during infancy, childhood, or adolescence.

There is insufficient evidence to evaluate the long-term effects in humans after developmental exposure (in utero through young adulthood) to hydroxyurea at therapeutic doses.

Evidence is sufficient to conclude that hydroxyurea produces developmental toxicity in rat fetuses from dams exposed orally to 200 mg/kg bw/day on GD 7–20 or 300 mg/kg bw/day on GD 6–15 as manifested by increased malformation rate, decreased body weight, and a decrease in number of live pups. Evidence is sufficient to conclude that hydroxyurea produces developmental toxicity in rat pups born to dams treated during gestation (GD 9–12) with 100 mg/kg bw/day i.p. manifested by an increase in malformations and alterations in behavior. The Expert Panel considers the studies using oral and i.p. administration to be corroborative.

Evidence is sufficient to conclude that hydroxyurea produces developmental toxicity in mouse pups born to dams treated during gestation (GD 6–17) with 200 mg/kg bw/day by gavage as manifested by increased malformation rate, decreased body weight, and increased resorptions and stillbirths.

There are insufficient data for an evaluation of the developmental effects of hydroxyurea treatment of immature experimental animals and of postnatal effects of hydroxyurea exposure during gestation.

The experimental animal data are assumed relevant to the assessment of human risk.

Note: The definitions of the term sufficient and the terms assumed relevant, relevant, and not relevant are in the CERHR guidelines at http://cerhr.niehs.nih.gov/news/guidelines.html.

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human

4.1.1 Female. **Rustin et al. (1984)**, supported by the Cancer Research Campaign, the Medical Research Council of England, and Lederle Laboratories, reported on the attainment of pregnancy after chemotherapy for gestational trophoblastic neoplasia. Women were sent questionnaires 2–22 years after completing therapy to identify pregnancies that had occurred since treatment. Of 457 survivors located, 440 returned completed questionnaires. There were 69 women whose therapy included hydroxyurea of whom 49 had not tried to conceive, three had tried but failed to conceive, three had conceived but did not have a live birth, and 14 had at least one live birth. Malformation rates for all pregnancies in the 457 survivors were reported not to significantly exceed general population rates; outcome information and conclusions specific to hydroxyurea were not provided.

Strengths/Weaknesses: The adverse health outcome was not well defined and may not have been appropriately measured. The exposure was not well-defined or appropriately measured. There appear to have been no controls. Potential confounding factors and effect modifiers were not identified. There was no evidence of a dose-effect relationship. Statistical methods were not clear. The power of the study was not adequate to detect an association of the size expected.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Bower et al. (1998), support not indicated, compared age of menopause onset in women who did or did not receive chemotherapy for gestational trophoblastic neoplasia. Women who had been seen in the authors' unit were sent a postal questionnaire 1.4–34 years after their treatment. Women who had received chemotherapy were divided according to chemotherapy regimen. The hydroxyurea-treated women (n = 299 evaluable subjects) also received mercaptopurine and either etoposide or actinomycin-D. There were 327 evaluable women who did not

Table 65
Summary of Developmental Toxicity in Mouse Studies

Strain, route	Endpoint	Dose (mg/kg bw or mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
NMRI, gavage GD 6–17	↑Stillbirth	—	≤200	Insufficient data for modeling				Roll and Bär (1969)
	↑Mortality during lactation period	—	≤200	Insufficient data for modeling				
	↓Birth weight	—	≤200	Insufficient data for modeling				
	↓Pups delivered	—	≤200	Insufficient data for modeling				
	↑Midterm resorption	—	≤200	Insufficient data for modeling				
	↓Fetal weight	—	≤200	Insufficient data for modeling				
NMRI, i.p. GD 11	↑Malformations, per fetus	250	300	213	188	—	—	Platzek and Schwabe (1999)
CD-1, i.p. GD 13	↓Body weight	—	≤400	Insufficient data for modeling				Woo et al. (2004)
	↓Cerebral cortical thickness	—	≤400	Insufficient data for modeling				
	↓Postweaning body weight gain	—	≤400	Insufficient data for modeling				
	↓Relative organ weight, males							
	Brain	—	≤400	721	593	568	455	
	Lung	—	≤400	Models not satisfactory				
	Left kidney	400	800	1207	793	1352	869	
	Spleen	—	≤400	340	294	439	366	
	Testis	—	≤400	532	426	671	521	
	Epididymis	—	≤400	Models not satisfactory				
	↓Relative organ weight, females	400	800					
	Brain	—	≤400	908	678	885	646	
	Lung	400	800	858	661	1011	822	
	Right kidney	400	800	707	584	739	616	
	Left kidney	400	800	787	694	788	697	
	Intestine	—	≤400	Models not satisfactory				
	Ovary	400	800	692	667	695	688	
	Uterus	400	800	644	563	760	699	
	Kinked tail	400	800	507	395	—	—	
	Microcephaly	—	≤400	Models not satisfactory				Yan and Hales (2005)
CD-1, i.p. GD 9	↑Fetal death	400	500	Insufficient data for modeling				
	↑External/skeletal malformations	400	500	Insufficient data for modeling				
ICR, i.p. GD 11	↑Cleft palate	—	250	Single-dose study				Kwasigroch and Skalko (1985)

GD, gestational day; i.p., intraperitoneal.

receive chemotherapy. Median (range) age at menopause was 49 (25–56) in the group that had received hydroxyurea and 53 (40–57) in the group that had not received chemotherapy. **[The authors did not statistically compare the hydroxyurea-exposed group to the unexposed group; however, Kaplan-Meier menopause-free survival plots were shown for the chemotherapy groups. The plot for the hydroxyurea-exposed group nearly overlay that for a group receiving methotrexate monotherapy, for which Mantel-Cox log-rank testing showed a significant difference from the unexposed group at $P < 0.03$.]** The authors concluded that chemotherapy for gestational trophoblastic neoplasia is associated with earlier age at onset of menopause.

Strengths/Weaknesses: The adverse health outcome was well defined but may not have been measured appropriately. The exposure was well-defined but not well measured. The controls were appropriate. Potential confounding factors and effect modifiers were not identified. There was no evidence of a dose-effect relationship. Statistical methods were not clear and probably not appropriate. The power of the study was adequate to detect an association of the size expected.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Pajor et al. (1991), support not indicated, presented a series of pregnancies in survivors of acute lymphoid leukemia and lymphoma. One woman who had been exposed to hydroxyurea and eight other agents had normal pregnancies 2 and 4 years after therapy. Her children were examined by dysmorphologists at ages 7 and 5.5 years, and no abnormalities were noted.

Strengths/Weaknesses: The exposure was not well defined or measured appropriately. Potential confounding factors were not identified. There was no evidence of a dose-effect relationship. The power of the study was not adequate to detect an association of the size expected.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Table 66
Summary of Developmental Toxicity in Single Dose-Level Rat Studies

Strain, route	Dose (mg/kg bw/day)	Significant developmental findings	Reference
F344, gavage	200 on GD 7–20	Dams: ↓Hematocrit Fetuses: ↓Body weight ↓Crown-rump length ↓Reticulocyte count and hematocrit ↑Erythrocyte size ↑Litters with malformations	Price et al. (1985b)
F344, gavage	200 on GD 7–20	Dams: ↓Body weight gain ↑Resorptions ↓Hematocrit ↑Mean corpuscular volume Fetuses: ↓Body weight ↓Crown-rump length ↓Placental weight ↓Relative spleen weight Pups: ↓Live pups at birth ↑Pups with malformations ↓Birth weight in males ↓Body weight during postnatal period ↓Relative weight of liver and spleen ↓Relative testis weight, PND 60 Delayed vaginal opening Delayed testis descent Delayed wire-grasping ability	Price et al. (1985a)
F344, i.p.	500 on GD 11	Dams: ↓Body weight gain Fetuses: ↓Body weight and length (both sexes) ↑Resorptions ↑Anomalies and variations	DePass and Weaver (1982)
Wistar, i.p.	500 on GD 11	Dams: No effect Fetuses: ↓Body weight and length (both sexes) ↑Resorptions ↑Anomalies and variations	
F344, i.p.	500 on GD 11	↑Fetal malformations	Maronpot et al. (1983)
Sprague-Dawley, i.p.	750 on GD 8	↑Postimplantation loss ↓Fetal weight ↑Malformations	Giavini et al. (1979)
	750 on GD 9	↑Postimplantation loss	
	750 on GD 10	↑Postimplantation loss ↓Fetal weight ↑Malformations	
	750 on GD 11	↑Postimplantation loss ↓Fetal weight ↑Malformations	
	750 on GD 11	↓Fetal weight ↑Malformations	
	750 on GD 13	↑Malformations	
	740 on GD 14	↑Malformations	
Wistar, i.p.	1,000 on GD 12 or 13	↑Limb malformations	Sugrue and DeSesso (1982)
Wistar, i.p.	500 on GD 11	↑Malformations	Chaube and Murphy (1973)
Wistar, i.p.	500 on GD 12	↓Fetal body weight ↑Resorptions ↑Malformations	Ritter et al. (1982)

Table 66
Continued

Strain, route	Dose (mg/kg bw/day)	Significant developmental findings	Reference
Sprague-Dawley, "injected"	150 on GD 6, 9, 12, 15, or 18	↑Hydrocephalus and microphthalmia after exposure on GD 9	Brunner et al. (1978)
Sprague-Dawley, i.p.	550 on GD 12	↓Pup body weight in lactation period ↓Female pup body weight PND 45 Delayed auditory startle reflex ↓Swimming ability ↓Rearing frequency in open field	Vorhees et al. (1979)
Sprague-Dawley, i.p.	2,000 on GD 14	↑Postnatal mortality ↓Wall climbing	Fritz and Hess (1980)
Sprague-Dawley, i.p.	550 on GD 12	↑Pup mortality PND 1–21 ↑Postweaning ambulation Delayed vaginal opening	Vorhees et al. (1983a)
Sprague-Dawley, i.p.	50 on PND 2–10	↓Pre- and postweaning pup weight Delayed swimming development Delayed vaginal opening ↓Running wheel activity ↓Cerebellar weight	Vorhees et al. (1983b)
Sprague-Dawley, s.c.	160 on GD 17–20	No effect on puberty onset, estrous cycles, or fertility of offspring	Gupta and Yaffe (1982)

GD, gestational day; i.p., intraperitoneal; PND, postnatal day; s.c., subcutaneous.

Table 67
Summary of Developmental Toxicity in Cat, Rabbit, and Hamster Studies

Species, strain, route	Dose (mg/kg bw/day)	Significant developmental findings	Reference
Cat, oral capsules	50 or 100 on GD 10–22	↑Resorptions at the high dose level ↓Fetal body weight at the high dose level	Khera (1979)
Rabbit, New Zealand White, s.c.	750 on GD 12	↑Resorptions ↓Fetal body weight ↑Malformations	DeSesso and Jordan (1977)
Rabbit, New Zealand White, s.c.	500 or 750 on GD 12	Alterations in craniofacial microvasculature within 4–9 minutes of dosing	Millicovsky and DeSesso (1980)
Rabbit, New Zealand White, s.c.	650 on GD 12	↑Resorptions ↑Malformations	DeSesso (1981a)
Rabbit, New Zealand White, s.c.	650 on GD 12	↓Fetal body weight ↑Malformations	DeSesso and Goeringer (1990a)
Rabbit, New Zealand White, s.c.	650 on GD 12	↓Fetal body weight ↑Malformations	DeSesso et al. (1994)
Hamster, Golden, i.v.	400–500 on GD 8	↑Embryo death	Ferm (1966)

GD, gestational day; s.c., subcutaneous; i.v., intravenous.

4.1.2 Male. Garozzo et al. (2000), support not indicated, reported in a Letter to the Editor that azoospermia developed in a 27-year-old man 6 months after hydroxyurea was started, with at least partial recovery of sperm count 11 months later (Fig. 6). Motility before therapy had been 75% and was 40% at the 11-month post-therapy session. The author concluded that reversible azoospermia may be associated with hydroxyurea therapy.

Strengths/Weaknesses: The exposure and outcome are of interest, but the weakness of this communication is that it represents a single case report.

Utility (Adequacy) for CERHR Evaluation Process: This letter is not useful in the evaluation process.

4.2 Experimental Animal

4.2.1 Female reproduction. Newton and Hayes (1968), support not indicated, examined the effect of hydroxyurea exposure on the rat ovary. Effects of triphenyltin acetate were also examined but will not be discussed here. At 42–46 days of age, female Holtzman rats were orally administered 40 mg/kg bw hydroxyurea. **[Hydroxyurea purity was not indicated. A control group**

was included but treatment of the control group was not discussed.] Three rats/group were killed at 4, 9, 14, 19, and 24 days after treatment for histopathologic evaluation of ovaries. Follicles at each stage of maturation were counted. Data were analyzed by ANOVA and least squares difference test. Values from the five sampling points were combined, apparently due to a lack of significant differences between the different time points. Hydroxyurea exposure did not significantly affect numbers of corpora lutea or secondary, tertiary, or mature follicles. The incidence of atresia was not increased in immature or mature follicles after hydroxyurea treatment. No "side effects" were reported in animals dosed with hydroxyurea. The study authors concluded that hydroxyurea had no apparent effect on ovulation.

Strengths/Weaknesses: The number of animals used in this study was too small, and a significant effect of treatment may have escaped detection as a result. There was no chemical verification of dosing preparations.

Table 68
Male Reproductive Effects in Mice Exposed to Hydroxyurea by Intraperitoneal Injection for 5 Days

	Hydroxyurea dose (mg/kg bw/day)			
Endpoint	625	1,250	2,500	5,000
Terminal body weight	↔	↔	↔	↓18%
Testes weight	↔	↔	↓41%	↓53%
Sperm count	↓38%	↓47%	↓60%	↓79%
Motile sperm	↔	↔	↓34%	↓59%

From Ficsor and Ginsberg (1980).
Benchmark dose modeling not possible due to lack of variances in the study results.
↓, Statistically significant decrease compared to controls; ↔, no statistically significant effects compared to controls.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process because the results suggest the study was under-powered.

Spencer et al. (2000), supported by NIH, examined the effect of hydroxyurea exposure on decidualization response in rats. A developmental toxicity assay was conducted and is described in Section 3.2.1.2. Pseudopregnancy was induced in Sprague-Dawley rats by vagino-cervical stimulation, and the day after the procedure was considered pseudopregnancy Day 1. Decidualization was induced by scratching the uterine epithelium on pseudopregnancy Day 4. Five rats/group were s.c. injected with saline vehicle or 500 mg/kg bw hydroxyurea (98% purity) on pseudopregnancy Days 5–8. Rats were killed on pseudopregnancy Day 9. Serum progesterone levels were measured by RIA. The other analyses were replicated three times in uterine endometrial tissue pooled from five dams. Protein concentrations were measured by the Lowry method, DNA concentration was determined by the Burton method, and alkaline phosphatase activity was measured by a spectrophotometry method. Capacity and numbers of estrogen receptors were assessed by radioligand binding. Nitric oxide synthase activity was determined by measuring conversion of ^{3}H-*l*-arginine to ^{3}H-*l*-citrulline and by a sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) method. Matrix metalloproteinases, a group of gelatinase enzymes, were quantified by SDS-PAGE. An RT-PCR technique was used to measure expression changes in mRNA for decidual prolactin-related protein and estrogen receptor in endometrium. Data were analyzed by Student's *t*-test and ANOVA.

Hydroxyurea exposure had no effect on serum progesterone levels or cytosolic estrogen receptor binding sites. Treatment with hydroxyurea resulted in >50%

Table 69
Summary of Male Reproductive Toxicity Studies in Experimental Animals

		Dose (mg/kg bw/day)		Reference
Model	Endpoint	NOAEL	LOAEL	
Holtzman rat, drinking water treatment × 70 days	↓Testicular weight	—	~460[a]	Mecklenburg et al. (1975)
	↑Abnormal tubules	—	~460[a]	
Sprague-Dawley rat, drinking water treatment × 3 months	↓Testis weight	—	~400[a]	Rich and De Kretser (1977)
	↓Caput epididymis weight	—	~400[a]	
	↓LH and FSH	—	~400[a]	
CF_1 mouse, treated i.p. × 5 days, evaluated 35 days later	↓Testis weight	1,250	2,500	Ficsor and Ginsberg (1980)
	↓Sperm count	—	≤625	
	↓Motile sperm	1,250	2,500	
C57B/6 × C3H/HeJF_1 mouse, treated i.p. × 5 days, evaluated 8 and 29 days later	↓Testis weight	25	50	Evenson and Jost (1993)
	↓Tetraploid germ cells	25	50	
	↓Haploid germ cells	50	100	
	↓Elongated spermatids	—	≤25	
	↑Chromatin denaturability	100	200	
ICR mouse, treated i.p. × 1 dose, evaluated 12 hr later	↑TUNEL-positive seminiferous tubules	—	≤100	Shin et al. (1999)
		—	—	
		—	—	

[a]Single-dose study.
None of these studies presented data in a manner suitable for benchmark dose modeling.

decreases in endometrial weight, protein, and DNA content. An 89% increase was observed for alkaline phosphatase activity in the hydroxyurea group. Reduced activities were observed for nitric oxide synthetase **[by ~27% compared to controls]** and matrix metalloproteinases **[by ~67%]** in the hydroxyurea group. Hydroxyurea treatment did not affect expression of estrogen receptor mRNA but induced a 30% decrease in expression of decidual prolactin-related protein mRNA. The study authors concluded that hydroxyurea induced adverse cellular and developmental responses in proliferative activities of endometrial cells that did not involve steroids or steroid receptors.

Strengths/Weaknesses: The evaluation of hydroxyurea effects on the deciduas as part of a developmental toxicity study is a strength.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process with results indicating that hydroxyurea has effects on cell proliferation in the endometrium.

4.2.2 Male reproduction. This section is arranged according to species. Within each section, oral exposure studies are presented before parenteral exposure studies and studies with multiple dose levels are presented before studies with single dose levels. The studies are then presented in order of publication.

4.2.2.1 Rat: Mecklenburg et al. (1975), support not indicated, used hydroxyurea to selectively delete germinal cells of male rats in a study focusing on regulation of follicle stimulating hormone (FSH) secretion. Eighteen sexually mature male Holtzman rats were given undosed drinking water and 90 rats were given tap water containing 3 mg/mL hydroxyurea **[purity not given]** for 70 days. **[Based on actual body weight at the end of the treatment period [0.392 kg] and a daily water intake of ~0.06 L (US EPA, 1988), CERHR estimated hydroxyurea intake at ~460 mg/kg bw/day.]** In the recovery phase of the study, no hydroxyurea was administered for 30 days. During the treatment and recovery phases, three treated animals/time period were killed at twice weekly intervals and four controls/time period were killed at monthly intervals. FSH and luteinizing hormone (LH) levels were measured in plasma, and testes were fixed in Bouin solution for a blind assessment of histopathology. Plasma FSH and LH levels were compared to negative controls and to 10 treated rats that were castrated on Day 49 of treatment. **[Statistical procedures were not discussed.]**

There were no clinical signs of toxicity or significant decreases in body weight in the hydroxyurea-exposed group. At the end of the 70-day treatment, testicular weights were significantly decreased **[by 55%]** in the hydroxyurea group. No effects were observed on the morphology of Leydig or Sertoli cells. Changes in the germinal epithelium were first noted after 14–17 days treatment. At that time, 10–20% of tubules contained a cell type that was located at the level of primary spermatocytes and had an eosinophilic cytoplasm and large dense nuclei. During the same time period, numerous large multinucleated eosinophilic cells were observed, and the numbers of multinucleated cells increased with duration of treatment. In rats treated for 21–70 days, histopathologic alterations were observed in 46/51 animals. Severity of histopathology increased with time and included cessation of spermatogonia division,

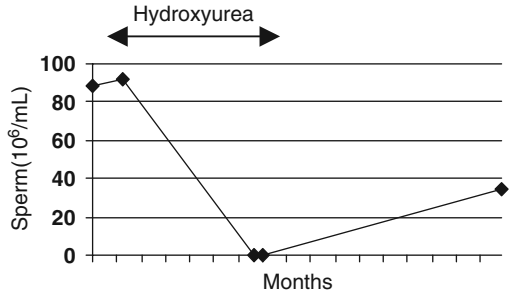

Fig. 6. Sperm count before, during, and after hydroxyurea therapy. Drawn using data from Garozzo et al. (2000).

presence of primary and secondary spermatocytes in the tubule lumen, reduction in tubule diameter, and presence of only Sertoli cells, spermatogonia, multinucleated cells, and scattered spermatids. Tubules from the most severely affected animals contained only Sertoli cells and Type A spermatogonia. Mitotic figures were generally lacking after 17 or more days of exposure. Meiotic figures were sporadically observed during the treatment period in the less severely affected animals. Mitotic and meiotic figures were observed frequently at several days into the recovery period and were present at control levels by 20 days after treatment stopped. Germinal epithelium was reestablished in >50% of tubules on Day 10 of recovery and in 95% of tubules by Day 30 of recovery. At some point during the recovery period, mature spermatozoa were observed in seminiferous tubule lumen. When compared to control rats, there were no consistent changes in plasma LH or FSH levels in the hydroxyurea-exposed group. Levels of FSH and LH in the hydroxyurea group remained lower than those of castrated rats. There were slight but significant increases in plasma LH levels in animals with the most severe testicular alterations, but a large overlap in LH levels was noted between groups with differing severity. The study authors concluded that hydroxyurea was well tolerated by rats and was effective in eliminating cells distal to Type A spermatogonia.

Strengths/Weaknesses: The masked, detailed evaluation of testicular histology is a strength. The single high dose level and the lack of reporting of water consumption levels are weaknesses.

Utility (Adequacy) of CERHR Evaluation Process: This study is useful in the evaluation process with results indicating that exposure to hydroxyurea produced reversible injury to the testicular germinal epithelium.

Rich and De Kretser (1977), supported by the National Health and Medical Research Council of Australia and the Ford Foundation, examined the effects of hydroxyurea exposure on the rat testis. The effects of vitamin A deficiency and irradiation were also examined but will not be discussed here. Over a period of 3 months, 60-day-old Sprague-Dawley rats were given drinking water containing hydroxyurea 0 or 3 g/L. **[Based on an assumed body weight of ~0.3 kg and water intake of ~0.04 L/day (US EPA, 1988), hydroxyurea intake was estimated at ~400 mg/kg bw/day. Hydroxyurea purity was not indicated.]** Rats were studied at 160–200 days of age. Testis and epididymis were weighed in 10 animals/

group. The testis was fixed in Bouin solution and examined histologically. Serum LH, FSH, and testosterone were measured by RIA in 8–26 animals/group. Androgen binding protein, a marker of Sertoli cell function, was measured in testicular and epididymal cytosol samples pooled from two groups of five animals; the protein levels were measured using ^{3}H-dihydrotestosterone and an electrophoresis method. Data were analyzed by ANOVA and Student's and Dunnett 2-tailed *t*-tests.

In the hydroxyurea group, testis weight was 40% and caput epididymis weight was **[49%]** of control values. Observations in testes from the hydroxyurea group included patchy spermatogenesis, marked reduction of seminiferous epithelium height, and dilated peritubular lymphatics. Leydig cell numbers and morphology were unaffected by hydroxyurea treatment. In the hydroxyurea-exposed compared to the control group, serum LH level was increased **[by 62%]** and serum FSH level was increased **[by 99%]**. There was no effect on serum testosterone level. Androgen binding protein in the testis was not affected by hydroxyurea exposure when expressed per mg protein; however, androgen binding protein per testis in the hydroxyurea group was reduced to 63% of the control value. In the caput epididymis of hydroxyurea-exposed animals, androgen binding protein was reduced by 33% when expressed per mg protein and by 74% when expressed as mg/testis. To determine if hydroxyurea reduced production of testicular androgen binding protein, accumulation of the protein in testis was measured after efferent duct ligation for 16 hr. In the hydroxyurea-treated group, the increase in androgen binding protein after efferent duct ligation was 31% of control values. The study authors concluded that hydroxyurea impaired Sertoli cell function.

Strengths/Weaknesses: The detailed assessments of testicular functional and structural endpoints are strengths. The single dose level of hydroxyurea and the lack of measurement of water consumption are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process with results indicating that exposure to hydroxyurea produced impairment of Sertoli cell function.

Rich et al. (1979), supported by the National Health and Medical Research Council of Australia and the Ford Foundation, examined the effect of hydroxyurea exposure on Leydig cell function. The effects of vitamin A deficiency and irradiation were also examined but will not be discussed here. Adult, 60-day-old, Sprague-Dawley rats were administered hydroxyurea in drinking water at 3 μg/L for 2.5 months. **[Based on the reported body weight of 300–350 g at the start of the study and an assumed water intake of ~0.04 L/day (US EPA, 1988), hydroxyurea intake was estimated at ~0.34–0.4 μg/kg bw/day. Hydroxyurea purity was not given.]** In eight rats/group, blood was collected before and 45 and 90 min after stimulation with 10 or 50 IU human chorionic gonadotropin (hCG). Serum testosterone was measured by RIA. Animals were killed, and testes from 80 control males and 126 hydroxyurea-treated males were fixed in Bouin solution for histopathologic evaluation by light and electron microscopy. **[Treatment of controls was not described. Time periods between the end of treatment and measurement of testosterone levels, and killing of rats were not reported.]** Data were analyzed by Dunnett 2-tailed *t*-test.

Hydroxyurea had no significant effect on basal testosterone level. In rats exposed to hydroxyurea, serum testosterone levels at 45 and 90 min and AUC for serum testosterone level after stimulation with either dose of hCG were significantly lower **[~20–25% lower at each hCG dose and time period]**. Exposure to hydroxyurea resulted in patchy regions of hypospermatogenesis. Changes in Leydig cells of treated rats included increased size of total cell, nucleus, and cytoplasm. Examination by electron microscopy showed increased amounts of smooth endoplasmic reticulum, Golgi complex, and mitochondria. The study authors concluded that Leydig cell dysfunction was shown but that cytological observations in Leydig cells indicated hypertrophy.

Strengths/Weaknesses: The detailed assessment of testicular functional and structural endpoints are strengths. The single dose level of hydroxyurea, the lack of measurement of water consumption, and the lack of clarity on timing and other aspects of experimental procedures are weaknesses.

Utility (Adequacy) of CERHR Evaluation Process: This study is useful in the evaluation process with results indicating that exposure to hydroxyurea produced modest impairment of Leydig cell function.

Baarends et al. (1995), supported by the Netherlands Organization for Scientific Research, examined the effects of hydroxyurea on rat testis, in a study focusing on the role of anti-müllerian hormone in testis development. Four adult Wistar rats **[apparently 2/group]** were i.p. injected three times at 16-hr intervals with saline vehicle or 500 mg/kg bw hydroxyurea **[purity not given]**. Rats were killed 5 days after the last injection. RNA was recovered from one testis to measure expression of mRNA for anti-müllerian hormone Type II receptor by RNase protection assay. The other testis was fixed in Bouin solution and examined histologically. Exposure to hydroxyurea resulted in loss of intermediate and Type B spermatogonia, preleptotene and leptotene spermatocytes, and most zygotene spermatocytes. No change in expression of mRNA for anti-müllerian hormone Type II receptor was observed in rats exposed to hydroxyurea. The authors concluded that specific types of cells regulating expression of anti-Müllerian hormone Type II receptor could not be identified by this study.

Strengths/Weaknesses: Weaknesses are the small number of animals, the single high hydroxyurea exposure level, and the lack of information on the effects of this high-dose treatment on the general condition of the animals.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Drug labels for hydroxyurea describe one fertility study that is not known to be available publicly. Male rats given 60 mg/kg bw/day hydroxyurea (~0.3 times the maximum recommended human daily dose on a mg/m^2 basis) through an unspecified route experienced testicular atrophy, reduced spermatogenesis, and decreased ability to impregnate females (Bristol-Myers-Squibb, 1999, 2001a,b; 2002, 2004, 2005a).

4.2.2.2 Mouse: Wyrobek and Bruce (1975), supported in part by the Medical Research Council and National

Cancer Institute of Canada, examined the effect of hydroxyurea exposure on sperm abnormalities in mice. Numerous other compounds were also examined but will not be discussed here. At 11–14 weeks of age, 4 C57BL × C3H/Anf or C57BL/6 × C3H/He mice **[an assumed four mice/group]** were i.p. injected with hydroxyurea **[purity not given]** in distilled water vehicle at 0 or ≤500 mg/kg bw/day for 5 days. **[Actual hydroxyurea doses were not reported and had to be estimated from Figure 2 of the study.] Mice were killed at 1, 4, and 10 weeks after exposure and sperm abnormalities were examined. [Statistical analyses were not conducted.] Sperm head abnormalities were increased, with the greatest incidence of effect observed at 4 weeks after hydroxyurea exposure. [At 4 weeks after hydroxyurea exposure, the greatest increase in sperm head malformations was observed around the mid-dose range of ~100–300 mg/kg bw/day. Increases in sperm head abnormalities at 10 weeks were dose-related, with the highest percentage observed at the high dose (~500 mg/kg bw/day).]** Banana-shaped head represented 46% of the sperm abnormalities associated with hydroxyurea at 4 weeks after exposure. The study authors concluded that the effect of hydroxyurea seemed to disappear within 10 weeks of exposure.

Strengths/Weaknesses: The lack of specification of hydroxyurea dose and lack of information on the general condition of the animals after treatment are weaknesses.

Utility (Adequacy) for CERHR Evaluation: This study is not useful in the evaluation process.

Lu and Meistrich (1979), supported by the National Cancer Institute and the Department of Health, Education, and Welfare, examined the effects of hydroxyurea exposure on mouse testicular cells. Numerous other compounds were also examined but will not be discussed here. Adult (8–10-week-old) C3HHeB/FeJ mice were given single i.p. injections of hydroxyurea **[purity not given]** at 7–7000 mg/kg bw. **[Individual doses were not reported.]** The dose range was selected to include the LD_{50} for mice. Control animals received a saline injection. **[It was not clear if saline was administered by i.v. or i.p. injection, because both types of injections were used, depending on compound.]** Eleven days after injection, one mouse/dose was killed, and testes were fixed in Bouin solution for staging of seminiferous epithelium to determine the types of cells killed. Twenty-nine days after injection, six mice from the control group and three mice from the hydroxyurea group were killed for counting of sperm heads as an indication of differentiated spermatogonia survival. Fifty-six days after treatment, six mice from the control group and three mice from the hydroxyurea group were killed, and stem cell survival was determined by sperm head count, lactate dehydrogenase activity (X-isozyme), and cell counts in testicular tubules. **[Methods of statistical analyses were not discussed.]**

At 11 days after treatment with 350–3500 mg/kg bw hydroxyurea, there was partial killing of A_1 spermatogonia through preleptotene spermatocytes, and the study authors indicated that the effect was consistent with hydroxyurea acting during the S-phase of the cell cycle. Complete killing of spermatogonia was observed at higher doses of hydroxyurea **[doses at which complete killing was observed were not clearly specified, but it is possible that it occurred at ≥1300 mg/kg bw/day]**. In sperm head counts conducted 29 days after hydroxyurea treatment, a plateau region was observed in the survival curve between two regions of decreased sperm counts, and the study authors indicated that it was due to killing of two populations of differentiated spermatogonial cells with different sensitivities. **[Decreases in sperm head counts were observed at hydroxyurea doses of ~10–200 mg/kg bw/day and at doses exceeding ~2000 mg/kg bw.]** The LD_{50} for differentiated spermatogonia was reported at 100 mg/kg bw. In the evaluation of stem cell survival 56 days after injection, hydroxyurea at doses of 7–7000 mg/kg bw induced small but statistically significant decreases in sperm head counts (94.3% of control levels). **[There did not appear to be a dose-response relationship at doses below ~2000 mg/kg bw.]** Changes in lactate dehydrogenase activity were reported to parallel effects on sperm head counts. **[Data were not shown.]** Complete spermatogenesis was observed in tubular cross sections 56 days after exposure to hydroxyurea. The LD_{50} for stem cells was reported at >5000 mg/kg bw. The study authors concluded that differentiated spermatogonia were the most sensitive of the testicular cells examined, likely related to their short mitotic cycle. The LD_{50} of 100 mg/kg bw in mice for differentiated spermatogonia was converted to an equivalent surface area dose of 330 mg/m^2 in mice and 10,000 mg/m^2 in humans. It was noted that spermatocytes past the preleptotene phase and spermatids were resistant to killing by hydroxyurea. The study authors indicated that if humans are as sensitive to hydroxyurea as mice, treatment of humans with hydroxyurea could result in transiently reduced sperm counts and infertility.

Strengths/Weaknesses: Strengths of this study are the evaluation of multiple dose levels, the detailed consideration of germ cell types, and the determination of a LOAEL for the most sensitive cell type. Weaknesses are the small number of animals, the absence of dose-response relationship for some of the endpoints, and the lack of reported statistical analysis. It is possible that the germ cell was considered the analytical unit rather than the individual male, which may have led to erroneous conclusions about the significance of the results.

Utility (Adequacy) of CERHR Evaluation Process: This study is not useful in the evaluation process.

Ficsor and Ginsberg (1980), supported in part by a Faculty Research Fund Grant, examined the effect of hydroxyurea exposure on sperm motility in mice. The effects of mitomycin C were also examined but will not be discussed here. At 10 or 12–16 weeks of age, three or four CF_1 male mice/group were i.p. injected for 5 days with hydroxyurea **[purity not given]** at 0 (saline vehicle), 625, 1250, 2500, or 5000 mg/kg bw/day. Animals were weighed during the treatment period and killed 35 days after the last injection for measurement of testicular weight and blind assessment of sperm count and motility. Data were analyzed by least significant difference test. Results are summarized in Table 68. Exposure to hydroxyurea resulted in decreased sperm counts at all dose levels. Testicular weight and sperm motility were decreased at ≥2500 mg/kg bw/day. Terminal body weight was reduced at the high dose. No visible signs of toxicity were observed in mice. The study authors concluded that their findings show the bioavailability of hydroxyurea or its metabolites in mammalian germ cells.

Strengths/Weaknesses: Strengths of this study are the use of multiple dose levels to permit evaluation of dose-response relationships and the evaluation of body weight and clinical condition. The small number of animals and lack of a NOAEL are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process with results indicating that exposure to hydroxyurea 625 mg/kg bw/day reduced spermatogenesis.

Evenson and Jost (1993), supported by March of Dimes and the National Science Foundation, examined the effects of hydroxyurea exposure on mouse testicular cells. At 13–15 weeks of age, ≥6 C57B/6 × C3H/HeJF$_1$ mice/group were i.p. injected with phosphate-buffered saline vehicle or hydroxyurea **[purity not given]** at 25, 50, 100, 200, 400, or 500 mg/kg bw/day for 5 days. Three mice/group/time period were killed at 8 and 29 days after exposure. Body and testis weights were measured. Testicular cell suspensions were prepared, and sperm was collected from epididymis. Testicular cells were stained with acridine orange and examined by flow cytometry. Abnormality in sperm was assessed using a chromatin structure assay that assessed shifts in fluorescence induced by chromatin damage. Data were analyzed using General Linear Model and correlation procedures. **[Although not always clearly indicated by authors, most of the findings presented appeared to attain statistical significance at levels of $P < 0.01$ or 0.05. Data were not presented in a manner that permitted benchmark dose modeling.]**

Hydroxyurea exposure had no effect on body weight. Testis weight was reduced significantly in the 400 and 500 mg/kg bw/day groups beginning 8 days after exposure. By 29 days after exposure, testis weight was reduced in groups exposed to ≥50 mg/kg bw/day and a 50% reduction compared controls was observed in the 500 mg/kg bw/day group.

Flow cytometry analyses showed a slight increase in percent haploid cells at ≥100 mg/kg bw/day and a concurrent decrease in percent tetraploid cells, which were nearly depleted at doses ≥400 mg/kg bw/day by 8 days after exposure. Also observed at 8 days post-exposure was a significant increase in percentage of elongated haploid cells. By 29 days after exposure, percentages of haploid cells decreased in groups exposed to ≥100 mg/kg bw/day and reached 50% of control values at the 500 mg/kg bw/day dose. Increases were observed for percentages of diploid cells at ≥200 mg/kg bw/day and tetraploid cells at ≥50 mg/kg bw/day 29 days after exposure; the increases were more than double compared to controls at the high dose. Effects on specific haploid cell populations 29 days after exposure were reported. Percentages of round spermatids were increased at all doses, with the maximum effect (64% increase) obtained at the 100 mg/kg bw/day dose. Percentages of elongating spermatids were increased in groups exposed to ≥200 mg/kg bw/day. Percentages of elongated spermatids were decreased **[apparently at all doses]** and were virtually eliminated at ≥200 mg/kg bw/day. According to the study authors, affected tetraploid cells 9 days after exposure were likely early and late pachytene spermatocytes (based on cell size). The study authors stated that the effects at 29 days represented germ cell renewal.

Eight days after hydroxyurea exposure, no effect was observed on the sperm population, as indicated by sperm chromatin structure assay and head morphology. Sperm chromatin structure was affected 29 days post-exposure. Distribution of α_t, a shift from green to red fluorescence that indicates increased chromatin susceptibility to acid or heat-induced denaturation, was elevated at 400 mg/kg bw/day. The standard deviation for α_t, which defines the extent of chromatin abnormality, attained statistical significance at doses ≥100 mg/kg bw/day. Percentage of cells outside the main population of α_t was increased at ≥200 mg/kg bw. At 29 days after exposure, the percentages of abnormal sperm head and sperm with detached head were increased **[apparently at ≥200 mg/kg bw/day]**, with maximum response obtained at 400 mg/kg bw/day.

According to the study authors, ''The major conclusions reached are that [hydroxyurea] inhibits DNA synthesis, probably by inhibiting ribonucleotide reductase, causing maturation depletion of pachytene spermatocytes and, subsequently, depletion of meiotic daughter cells and differentiated cell types leading to mature sperm. This inhibition of DNA synthesis is related to an alteration of sperm chromatin structure and abnormal sperm head morphology.''

Strengths/Weaknesses: Strengths are the use of flow cytometry to evaluate testicular germ cell populations, the use of multiple doses of hydroxyurea, the use of two assessment times, and the reporting of testis and body weight. The lack of correlation of the germ cell endpoints with fertility endpoints is a weakness. Twenty-nine days is not enough time to allow reversal of hydroxyurea effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process with results indicating that effects of hydroxyurea on cells in the testicular germinal epithelium appear to depend on decreased DNA synthesis. The LOAEL was 50 mg/kg bw/day based on reduced testis weight at one time point (NOAEL 25 mg/kg bw/day), but it is not clear that this effect would result in a change in fertility. Sperm changes that would like result in at least a transient decrease in fertility were seen at 200 and 400 mg/kg bw/day.

Wiger et al. (1995), support not indicated, examined the effects of hydroxyurea exposure of mice on spermatogenesis and sperm chromatin structure. The effects of acetaminophen were also examined but will not be discussed here. A series of experiments were conducted in adult (6–8 week-old) B6C3F$_1$/BOM adult male mice. Data were analyzed by Student's *t*-test. **[Protocol details were very limited. In some cases the mice were exposed by i.p. injection, and i.p. is assumed to have been the route in all experiments. The numbers of animals treated and examined were not always reported. Available information is presented below.]**

In one experiment, five mice/group were i.p. injected with 200 mg/kg bw/day hydroxyurea **[purity not given]** for 5 days. At 5 or 10 days after the last treatment, liver, testis, and caput epididymis were fixed in 2% neutral glutaraldehyde and examined for histopathologic effects. Diameters of 20 seminiferous tubules/testis were measured in two mice from the control group and three mice from the hydroxyurea group. Five days after hydroxyurea treatment, a number of atrophied tubules with reduced diameters, large vacuoles, and few cells were observed. In the hydroxyurea groups, tubule diameters

were 91.4% of control values ($P \leq 0.003$). Large "non-sperm" cells were observed in tubules of the caput epididymis. Ten days after treatment, atrophic areas in the testis were observed in half the mice from the hydroxyurea-exposed group. Some of the tubules were empty, collapsed, or contained mostly Sertoli cells. Five and 10 days after treatment, livers of mice from the hydroxyurea group had extensive intracellular vacuolization that did not stain positive for glycogen.

In a second experiment, five mice/group were i.p. injected with 0 (phosphate-buffered saline [PBS]), 100, or 200 mg/kg bw/day hydroxyurea for 5 days. At various time points, mice were injected with ^{3}H-thymidine and killed 1 hr later to determine thymidine incorporation into testicular DNA. After the last treatment, thymidine intake was reduced by ~65% compared to controls in the 100 mg/kg bw/day group and was blocked nearly completely in the 200 mg/kg bw/day group. In a time-response study conducted in the 200 mg/kg bw/day group, thymidine intake was ~8% of control levels 4 hr after exposure **[3 hr according to Figure 4 of the study]** and returned to control levels 20 hr after exposure.

In a third experiment, various endpoints were examined after exposure of mice to hydroxyurea for 5 days. Body and testis weights were examined in two to four mice/group treated with 0 or 200 mg/kg bw/day hydroxyurea. Animals were weighed for up to 45 days after treatment. Testes were weighed on Days 27 and 33 after treatment. Testicular and sperm cells were examined by flow cytometry and acridine orange staining at 26 days after exposure to hydroxyurea by i.p. injection at 0, 100, 200, or 400 mg/kg bw/day. Vas deferens sperm chromatin structure was examined using an acridine orange staining method after exposure of mice to 0 (n = 8) or 200 mg/kg bw/day hydroxyurea (n = 3 or 4).

No treatment-related increases in mortality were observed. In contrast to control mice, mice exposed to hydroxyurea did not gain weight during the first 5 days after treatment. At later time points, body weight gain did not differ from controls for up to 45 days. Testis weights were ~40–45% lower than control values on Days 27 and 33 after treatment ($P < 0.05$). The number of tetraploid cells was decreased at 5 and 10 days after treatment. By 26 days after treatment with hydroxyurea 200 mg/kg bw/day, there were dose-related decreases in numbers of haploid cells and increases in numbers of diploid and tetraploid cells. **[Apparently, statistical significance was not achieved at lower dose levels]**. The tetraploid cells affected were reported to be predominantly early pachytene spermatocytes. Among the haploid cells, there were dose-related increases in numbers of round cells and decreases in numbers of elongated cells. **[Figures in the study showed conflicting findings for elongating cells. Figure 6 showed a decrease in elongating cells at 26 days, whereas Figure 7 showed an increase in the same cells at 26 days.]** By 45 days after exposure, ratios of the different cell types returned to normal. Exposure to hydroxyurea increased significantly the number of sperm with abnormal chromatin structure at 5, 27, and 33 days after exposure. **[A 511% increase compared to control values was observed by 33 days after exposure.]**

The study authors concluded that high doses of hydroxyurea inhibit testicular DNA synthesis, which leads to reduced testicular weight, reduced numbers of early pachytene spermatocytes, changes in proportion of cells in various spermatid stages, and an apparent alteration in sperm chromatin structure.

Strengths/Weaknesses: The strength of this study is the sophisticated methodology applied to the evaluation of testicular effects of hydroxyurea. This strength is offset by inadequate reporting of methods and results, which detracts from the reliability of any conclusions that might have been drawn from this work.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Shin et al. (1999), supported by the Japanese Ministry of Education, Science, Sports, and Culture, examined the effects of hydroxyurea exposure on apoptosis in testis of adult ICR mice (6–7 weeks old). In a time-response study, male mice were assigned randomly to groups and i.p. injected with saline vehicle or 400 mg/kg bw hydroxyurea. Mice were killed, and testes were evaluated at 0, 4, 8, 12, 18, 24, and 48 hr after hydroxyurea treatment. In a dose-response study, mice were exposed to hydroxyurea **[purity not given]** at 0, 100, 200, or 400 mg/kg bw and killed 12 hr later. In both sets of studies, one testis was used for examination of apoptosis by the TUNEL method and the other was used for examination of DNA fragmentation using the ligation-mediated PCR method. Seminiferous tubules were also staged. **[Numbers of animals treated and examined were not reported by the study authors.]** Statistical analyses included ANOVA followed by post-hoc Scheffé test.

Treatment with hydroxyurea did not affect body or testis weights. **[Data were not shown.]** There were no treatment-related clinical signs of toxicity or deaths observed. In both the control and hydroxyurea groups, apoptosis was observed in spermatogonia and spermatocytes, but numbers of apoptotic cells were increased by hydroxyurea exposure. Dose-response studies showed increases in percentages of tubules containing TUNEL-positive cells at ≥100 mg/kg bw, numbers of TUNEL-positive cells in evaluated tubules at ≥200 mg/kg bw, and DNA fragmentation in tubules at 400 mg/kg bw. Apoptosis was not observed in multinucleated giant cells in damaged tubules. In time-course studies, DNA fragmentation, percentages of tubules containing TUNEL-positive cells, and numbers of TUNEL-positive cells in tubules peaked 12 hr after exposure to 400 mg/kg bw hydroxyurea. Increases in apoptotic tubules were observed in Stages I–III, IV–VI, and X–XII. **[Data were presented graphically and were not suitable for benchmark dose modeling.]** The study authors concluded that hydroxyurea induced a dose- and stage-dependent increase in testicular germ cell apoptosis.

Strengths/Weaknesses: The time-course and dose-response elements of this study, the evaluation of apoptosis by stage, and consideration of body and testis weights are strengths. Lack of indication of the number of animals and lack of evaluation of fertility parameters are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study identifies 100 mg/kg bw as an effect level for an increase in apoptosis in the mouse seminiferous tubule, but the lack of correlation with fertility endpoints decreases the utility of this observation.

Chandley et al. (1977), support not indicated, showed hydroxyurea-induced inhibition of DNA synthesis in a study examining meiosis in mouse spermatogenic cells.

Within a period of 18–24 hr, Swiss mice were i.v. injected three times with 1 M hydroxyurea **[~500 mg/kg bw, based on volume injected and an assumed body weight of ~0.03 kg (US EPA, 1988). Hydroxyurea purity was not given.]**. Mice also received ^{3}H-thymidine at the time of the last two injections. A control group was included, but treatment of that group was not described. Total radioactivity levels were measured by scintillation counting and autoradiographs were prepared for spermatogenesis fractions. Within 24 hr of exposure to hydroxyurea, total S-phase radioactivity in spermatogenic cells was negligible in treated compared to control mice. Examination by autoradiography showed lower percentages of labeled cells and reduced grain count/cell. The study authors stated that hydroxyurea suppressed semi-conservative meiotic DNA synthesis.

Strengths/Weaknesses: Weaknesses are the lack of specification of animal number, lack of clarity on hydroxyurea dose, the probably very high dose level, and the lack of information on fertility endpoints. The absence of statistical analysis is a weakness, offset to some extent by the large changes in the hydroxyurea-exposed cells.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Dietrich et al. (1983), support not indicated, reported some information on the effects of hydroxyurea on spermatogenesis in mice in a study focusing on development of testicular cell culture methods. In three sets of experiments that were duplicated, Swiss mice (6–15 weeks old) received three i.p. injections of 350 mg/kg bw hydroxyurea **[purity not given]** in saline at 12-hr intervals. The injection procedure was repeated 6 days later. Mice were killed 10–14 days after the last injection and testicular cells were cultured. A hydroxyurea-induced gap in spermatogenesis was shown by the reappearance of pachytene, diplotene, or round spermatids, cells that were missing at the beginning of some culture periods, after 7–10 days in culture medium.

Strengths/Weaknesses: Weaknesses are the wide age range of the animals, the very large hydroxyurea dose, and the atypical treatment regimen. This study was not designed to characterize the reproductive toxicity of hydroxyurea.

Utility (Adequacy) of CERHR Evaluation Process: This study is not useful in the evaluation process.

van Buul and Bootsma (1994), supported by the Association of the CEC Radiation Protection Research Action and the University of Leiden, Netherlands, examined the effects of hydroxyurea exposure on chromosome damage and killing of mouse spermatogonial cells. At 10–16 weeks of age, Swiss mice were i.p. injected with 500 mg/kg bw hydroxyurea **[purity not given]** alone or in combination with an unspecified dose of 3-aminobenzamide. Additional mice received x-ray exposure 16 or 48 hr after hydroxyurea treatment. Control rats received the PBS vehicle. Reciprocal translocations in mouse spermatogonial stem cells from 3–12 mice/group were examined between 90–208 days after treatment. At 3 weeks after exposure, 3–12 mice/group were killed and testes were fixed in Bouin solution for determination of repopulation index (percentage of tubules showing spermatogenic repopulation with at least 1 spermatogonium). **[Statistical analyses were not reported.]**

Exposure to hydroxyurea alone or in combination with 3-aminobenzamide did not affect percentages of translocations. When hydroxyurea was administered at 16 hr before x-ray exposure, percentages of translocations were increased compared to exposure to x-rays alone. The repopulation index was 100% after exposure to hydroxyurea alone or in combination with 3-aminobenzamide. When x-ray exposure occurred after hydroxyurea treatment, the repopulation index was lower (i.e., greater cell killing occurred) compared to exposure to x-rays alone. A greater magnitude of effect was observed with x-ray exposure occurring 16 compared to 48 hr after hydroxyurea exposure. The study authors concluded that a high degree of cell killing and translocations occur when mice are x-rayed 16 hr after treatment with hydroxyurea.

Strengths/Weaknesses: The evaluation of x-irradiation interaction with hydroxyurea toxicity may be of interest mechanistically, but it does not provide useful information on the reproductive effects of hydroxyurea. In the combined treatment experiment with 3-aminobenzamide, the unspecified dose makes results of that experiment uninterpretable. The inclusion of a recovery component is a strength, but lack of effect of hydroxyurea treatment alone offsets the strength of the recovery observations.

Utility (Adequacy) of CERHR Evaluation Process: This study is not useful in the evaluation process.

Archibong et al. (2000), support not indicated, examined the effect of hydroxyurea treatment on spermatogenesis in mice. Adult male ICR mice were randomly assigned to groups and i.p. injected with 0 (saline vehicle) or 100 mg/kg bw/day hydroxyurea **[purity not given]** for 28 days. Six mice/group/time period were killed 24 hr and 1, 2, 3, and 4 months after the last dose. Endpoints evaluated included testis weight, sperm motility and density, and plasma LH, FSH, and testosterone levels. **[No details were provided about procedures for laboratory analyses or statistical evaluation of data.]** Effects observed after hydroxyurea exposure **[presumably at 24 hr after exposure]** were **[percent change compared to control]** decreases in testis weight **[67%]**, sperm density **[93%],** motile sperm **[78%]**, plasma testosterone level **[92%]**, and plasma LH level **[88%]**. Plasma FSH levels were increased **[by 164%]** 24 hr after hydroxyurea exposure. There was no effect on body weight. At 1–4 months after exposure, plasma FSH and LH levels remained lower than control values. Fertility indices **[presumably sperm endpoints, but not described]** gradually increased during the 4-month recovery period but remained lower than control value. **[Data were not shown.]** Litter size was reduced **[by 37%]** in females bred to hydroxyurea-treated males. **[No details were provided about the mating study, such as numbers of males examined and time period between mating and exposure. Results described above were reported to be statistically significant, but levels of significance were not reported in most cases.]** The study authors concluded that hydroxyurea appears to perturb reproductive efficiency of male mice through modulation of pituitary gonadotropins.

Strengths/Weaknesses: Strengths of this study are the relatively long treatment period, the long evaluation period, the examination of multiple endpoints of testicular function, the use of a treatment regimen that did not affect

body weight, and the inclusion of a mating trial. Weaknesses are the lack of experimental detail in this very short report, the use of a single hydroxyurea dose level, and the incomplete reporting of the statistical analysis.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process, because important information is missing from the report.

4.2.2.3 Other mammals: Singh and Taylor (1981), supported by the Public Health Service, examined the effects of hydroxyurea on hamster sperm. At 10–12 weeks of age, 6–9 Charles Lakeview hamsters (PD4 strain)/group were i.p. injected with 0 (distilled water vehicle), 10, 50, or 250 mg/kg bw/day hydroxyurea **[purity not given]** for 5 days. Two or three hamsters/dose were killed 1, 4, and 10 weeks after treatment **[1, 4, and 12 weeks after treatment in study figures and Results section]**. Body and testis weight were measured, and sperm was collected from cauda epididymis for assessment of sperm count and morphology. **[No statistical analyses were reported.]** Hydroxyurea treatment had no effect on sperm head abnormalities, with the exception of an increased incidence in the low-dose group at 12 weeks post-treatment. The study authors noted a progressive decrease in sperm count that occurred in all dose groups. **[Week 4 post-treatment was the only time point at which a dose-related reduction in sperm counts occurred; sperm counts in treated groups were ~95, 80, and 30% of control values at each respective dose. Non-dose-related decreases in sperm counts occurred in all hydroxyurea dose groups 1 week after treatment (~55, 45, and 78% of the control group in the low-, mid-, and high-dose group) and 12 weeks after treatment (10, 48, and 45% of control levels in the low-, mid-, and high-dose group)]. A decrease in testis weight [to ~20–62% of the control value]** occurred at the low dose during weeks 4 and 12 of the study, but testis weights remained constant or were increased **[to ~63–100% of control values]** at higher doses. During the first week of the study, body weights were higher than controls in all dose groups. A non-dose related decrease in body weight **[to ~80–90% of control values]** occurred by Week 12. The study authors concluded that hydroxyurea did not induce sperm abnormalities but did affect testis weight and sperm numbers adversely.

Strengths/Weaknesses: The use of multiple dose groups and multiple assessment times are strengths. The lack of a dose-response relationship for some of the endpoints and the variability in response over time are weaknesses, perhaps due to the variability in these endpoints among the small number of animals examined at each time point and dose.

Utility (Adequacy) of CERHR Evaluation Process: This study is not useful in the evaluation process.

Carnero et al. (1991), support not indicated, used hydroxyurea in the examination of meiosis in spermatocytes from the pine vole *Pitymys duodecimcostatus* (Rodentia, microtidae). In the main portion of the study, 16 males received three sets of hydroxyurea treatments. Each set was separated by a 3-day period. In each treatment set, the animals received three i.p. injections of 350 mg/kg bw hydroxyurea **[purity not given]** in PBS, with 12-hr intervals between injections. Groups of animals were killed every 4 days after injection. Synaptonemal complex preparations were examined by electron microscopy. Peak time for appearance of each substage included preleptotene at 4 days, leptotene at 7 days, zygotene at 8 days, zygotene pachytene at 11 days, mid pachytene at 14 days, and late pachytene at 16 days post-treatment. The study authors noted secondary peaks for each substage. They stated that secondary peaks occurring later in the cycle most likely represented cells that were arrested in the S-phase and were delayed in restarting the cycle. Secondary peaks occurring earlier in the cycle were thought to most likely represent cells arrested after completion of S-phase.

Strengths/Weaknesses: The attempt to examine substages of meiotic prophase and the use of an atypical model are strengths. The complicated treatment schedule of nine injections over 6 days was presented without rationale and resulted in a very high hydroxyurea exposure level. The sequenced examination of testes is difficult to coordinate with the authors' description of the treatment schedule, and it is unclear how many animals were examined at each time point. The presentation of data is largely qualitative, and there is no information that might be used to assess the fertility potential of treated males.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

4.2.2.4 In vitro studies: Lee and Suzuki (1981), support not indicated, examined the effects of hydroxyurea exposure on DNA synthesis in mouse spermatogenic cells. The goal of the study was to use hydroxyurea to examine potential effects on unscheduled DNA synthesis of methyl methanesulfonate, which will not be discussed here. Prepubertal spermatogenic cells from ~20-day-old CD-1 mice were cultured for 30 min in media containing hydroxyurea at 0, 1, 2.5, 5, 10, or 20 mM **[0, 70, 175, 350, 701, or 1401 mg/L]**. ^{3}H-thymidine was then added to the media, and incubation was continued for 1, 2, or 4 hr. Radioactivity levels were measured with a liquid scintillation spectrophotometer. Data were analyzed by Student's *t*-test. At all doses examined at each time period, hydroxyurea induced concentration-related reductions in DNA synthesis, as determined by radioactivity levels. At the highest hydroxyurea concentration, DNA synthesis was reduced by 90%. The study authors concluded that 20 mM **[1401 mg/L]** hydroxyurea induced incomplete inhibition of semiconservative DNA synthesis, but that the concentration was acceptable for use in studies of unscheduled DNA synthesis.

Strengths/Weaknesses: This study identifies suppression of DNA synthesis in vitro with exposure to high concentrations of hydroxyurea.

Utility (Adequacy) for CERHR Evaluation Process: The study is not useful in a consideration of reproductive effects.

Brock et al. (1983), supported by the National Science Foundation and the National Cancer Institute, examined the effects of hydroxyurea exposure on DNA and histone synthesis in spermatogenic cells and brain tumor cell lines. In in vitro studies, spermatogenic cells were obtained from adult and immature Sprague-Dawley rats and incubated in media containing ^{3}H-arginine, ^{3}H-lysine, and ^{14}C-thymidine with and without 50 μM

[3.5 mg/L and possibly 100 μM (7 mg/L)] hydroxyurea for 2 hr. Exposure to hydroxyurea in cell culture began 20 min before labeling. RT489 glioma tumor cells were examined as solid tumors and as a cell line. Tumor cells were exposed to media containing ^{3}H-arginine, ^{3}H-lysine, and ^{14}C-thymidine with and without 10 or 50 μM **[0.8 or 3.5 mg/L]** hydroxyurea for an unspecified time period. In the in vivo study, RT489 glioma tumors were induced in neonatal rats. At 20 days of age, the rats were i.p. injected with hydroxyurea **[purity not given]** at 0, 100, or 500 mg/kg bw. Hydroxyurea was administered at 10 and 70 min before and 50 min after injection of radioactively labeled arginine, lysine, and thymidine. Animals were killed 90 min after exposure to the radioactive label. In in vitro and in vivo studies, histones were extracted, separated electrophoretically, examined by spectrophotometry, and measured for radioactivity level. Protein was measured by the Lowry method, and DNA was quantitated by the Burton method. **[No statistical analyses were reported.]**

After in vitro exposure of spermatogenic cells to 50 μM **[3.5 mg/L]** hydroxyurea, synthesis of somatic or testicular histones was not inhibited in adult cells, and only a slight reduction in synthesis of the H4 histone was observed in cells from immature rats. **[Although the text indicated that DNA synthesis was inhibited at a hydroxyurea concentration of 100 μM (7 mg/L), no effect on DNA synthesis was evident in Figures 1 and 2 of the study, which only show the effect for the 50 μM concentration. The text of the study appears to suggest that DNA synthesis was inhibited at 100 μM hydroxyurea, but that little or no effects were observed on histone synthesis at ≤100 μM hydroxyurea.]** In the in vitro and in vivo studies of tumor cells, dose-related reductions were observed for both histone and DNA synthesis. Hydroxyurea did not affect total protein synthesis in the cells, indicating the effects were specific for histone synthesis. The study authors concluded that their results support the theory that a high degree of coupling between histone and DNA synthesis seems to occur only in proliferating, non-differentiating cells.

Strengths/Weaknesses: The attempt to link histone and DNA synthesis is a strength; however, the identification of such a link for tumor cells and not spermatogenic cells is a weakness. The discrepancy between the text and the figures is also a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation process.

4.2.3 Fertility study. Kissam and Hays (1966), support not indicated, examined the use of hydroxyurea as a chemosterilant for house flies (*Musca domestica*). The goal of the study was to find a hydroxyurea dose that reduced fertility without increasing mortality. Other compounds were also examined but will not be discussed here. Based on an initial study, a test dose of 5.0 g/L feed was selected for hydroxyurea and tested in four replicate randomized blocks. Each replicate was conducted over a 10-day period with 25 flies/sex/group. A control group was also used. **[Although treatment of that group was not described, it is assumed they received undosed feed.]** Reproduction was assessed by determining average numbers of larvae produced by living females on each day of the test. Data were analyzed by Duncan multiple range test. Compared to the control group, exposure to hydroxyurea resulted in decreased numbers of larvae/day **[91% reduction]** and total eggs deposited **[96% reduction]** ($P < 0.05$). Mortality rates of treated males and females did not differ significantly from control values. The study authors concluded that hydroxyurea is a promising agent of which further investigation is justified.

Strengths/Weaknesses: A weaknesses of this study is the description of a dosing regimen that cannot be converted for use in human risk assessment. The purpose of this study was to sterilize flies, not to evaluate effects on mammalian reproduction.

Utility (Adequacy) of CERHR Evaluation Process: This study is not useful in the evaluation process.

4.3 Utility of Reproductive Toxicity Data

There are no human studies that are useful in the assessment of the reproductive toxicity of hydroxyurea. There is one experimental animal study that can be used to assess female reproductive toxicity of hydroxyurea. This study involves the development of the decidua in pseudopregnant rats (Spencer et al., 2000). There are five experimental animal studies that provide useful information on male reproductive toxicity of hydroxyurea. Three of these studies used multiple dose levels and are suitable for a dose-response evaluation.

4.4 Summary of Reproductive Toxicity Data

4.4.1 Human studies. No human studies had utility for the evaluation process.

4.4.2 Experimental animal. Pseudopregnant Sprague-Dawley rats treated with hydroxyurea 500 mg/kg bw/day s.c. on pseudopregnancy days 5–8 showed a decrease in weight of the endometrium and decreased endometrial protein and DNA content (Spencer et al., 2000). These results suggested a decrease in decidual response.

Male reproductive toxicity studies in adult animals are summarized in Table 69. The two rat studies (Mecklenburg et al., 1975; Rich and De Kretser, 1977) used single dose levels of hydroxyurea and showed testicular toxicity at those doses (~400 mg/kg bw/day in drinking water). A multiple dose-level study in mice (Evenson and Jost, 1993) showed effects by flow cytometry assessment on testicular germ cell distribution beginning at hydroxyurea doses of 50 mg/kg bw/day. Evidence of apoptosis in the seminiferous epithelium was identified in mice 12 hr about a single i.p. treatment with hydroxyurea 100 mg/kg bw (Shin et al., 1999). None of these studies included an assessment of fertility.

Reversibility of histologic evidence of hydroxyurea toxicity for the rat seminiferous epithelium was shown 30 days after treatment (~460 mg/kg bw/day in drinking water) was stopped (Mecklenburg et al., 1975). Decreased testis weight and flow cytometric abnormalities in testicular germ cell distribution were still detected 29 days after treatment (50 mg/kg bw/day i.p.) in mice (Evenson and Jost, 1993). The changes in germ cell distribution were consistent with depletion of pachytene spermatocytes secondary to decreased DNA synthesis. Taken together, these two studies do not provide an adequate assessment of reversibility of testicular toxicity due to hydroxyurea administration.

Expert Panel Conclusions

There are no human data on the reproductive effects of hydroxyurea.

Evidence is insufficient for evaluation of female reproductive toxicity of hydroxyurea in experimental animals.

Evidence is sufficient to conclude that hydroxyurea produces reproductive toxicity in male mice at 50 mg/kg bw/day i.p. given for 5 days manifested by decreased testis weight and sperm count. Evidence is sufficient to conclude that hydroxyurea produces reproductive toxicity in male rats at ~400–460 mg/kg bw/day in drinking water for 70–90 days as manifested by reduced testis weight and histologic abnormalities of seminiferous tubules. There are no data on effects of hydroxyurea on male fertility in experimental animals.

The experimental animal data are assumed relevant to the assessment of human risk.

Note: The definitions of the term sufficient and the terms assumed relevant, relevant, and not relevant are in the CERHR guidelines at http://cerhr.niehs.nih.gov/reports/journal/CERHRguidelines.pdf

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

5.1.1 Development. Hydroxyurea therapy during pregnancy has been reported. There are insufficient data on the outcomes of offspring exposed to hydroxyurea during pregnancy or lactation. Three minor malformations were observed in a case series of 32 pregnancies in 31 women (Thauvin-Robinet et al., 2001). It is unclear whether these malformations were related to the use of hydroxyurea. There are no data on the long-term effects on offspring born to women exposed to hydroxyurea.

Reports on the use of hydroxyurea in children have almost exclusively involved therapy of hemoglobinopathies, usually sickle cell disease. Studies that evaluated growth (height and weight) and development did not identify delays in growth or pubertal progression; however, studies were limited by various durations of follow-up. There are insufficient data on effects on growth in children <5 years of age. There are no studies of reproductive function in individuals treated with hydroxyurea during childhood or adolescence. There are no studies on subsequent generations after the exposure of developing germ cells to hydroxyurea in utero or during infancy, childhood, or adolescence. There are no studies on the long-term health effects, including abnormal development and risk of malignancy, after childhood exposure to hydroxyurea.

Developmental toxicity has been assessed in laboratory animals after maternal treatment with hydroxyurea during gestation and after direct exposure of rat pups. These data are sufficient to conclude that hydroxyurea is a developmental toxicant in rats as indicated by increased malformation rate, decreased body weight and a decrease in the number of live pups after oral exposure of dams to 200 mg/kg bw/day on GD 7–20 or ~300 mg/kg bw/day on GD 6–15 (Aliverti et al., 1980). In addition, rat pups born to dams treated with 100 mg/kg bw/day i.p. from GD 9–12 exhibited an increase in malformations and alterations in behavior (Asano et al., 1983). Data are sufficient to conclude that hydroxyurea is a developmental toxicant in mice as indicated by an increase in malformation rate, a decrease in body weight, and an increased rate of resorptions and still births in mouse pups born to dams treated orally from GD 6–17 with 200 mg/kg bw/day. These data are assumed relevant to the assessment of potential human hazard. There are insufficient data for an evaluation of the developmental effects of hydroxyurea treatment of immature experimental animals. There are also insufficient data to evaluate other potential postnatal effects of hydroxyurea exposure during gestation.

Mice administered 200 mg/kg of hydroxyurea by either of two routes (i.p. or oral) excreted ~90% of the dose as hydroxyurea in urine at 24 hr after dosing (Table 2 in Adamson et al., 1965), suggesting that bioavailability is very high after oral or i.p. administration and only ~10% of the dose is metabolized. In another study (Van den Berg et al., 1994) clearance of hydroxyurea from plasma was measured for 1 hr after i.p. administration of 100 mg/kg bw in nude mice. Peak blood concentration was ~85 μg/mL at 10 min after dosing and the calculated plasma half life was 11 min.

As part of a human pharmacokinetic study, seven healthy adult humans were administered 15 mg/kg bw orally in capsules. Thirty-eight percent of the administered dose was recovered in the urine as hydroxyurea at 36 hr after dosing, suggesting that 62% of the dose is metabolized (Yan et al., 2005). The time to peak plasma level for these subjects ranged from 15 min to 1 hr and the measured peak plasma concentrations averaged 28 μg/mL. The calculated plasma half life was 3.1 hr (Table II in Yan et al., 2005).

If linear kinetics prevail, and the nude mice were dosed with 15 mg/kg bw (the human dose of hydroxyurea), the peak plasma concentration would be 13 μg/mL or about half of the human measured concentrations. This estimate suggests that the measured peak plasma concentrations of hydroxyurea associated with adverse effects in animals are similar to plasma concentrations of hydroxyurea achieved in humans on therapy.

In addition, the model of Beliles et al. (1991) (Table 8 of this report) estimated the same AUC in humans administered hydroxyurea at 10 mg/kg bw/day, which is at the low end of the therapeutic dose range, and rats administered 100 mg/kg bw/day, a dose at which developmental toxicity occurred in the study of Asano et al. (1983).

Although there are differences in factors affecting pharmacokinetics between animal species and humans, and between pregnant and non-pregnant individuals, blood concentrations of hydroxyurea are estimated to be similar in dams with adverse developmental outcomes and in patients on hydroxyurea therapy.

5.1.2 Reproduction. There are no human data.

Overall, experimental animal evidence is largely lacking with regard to reproductive endpoints in both sexes.

Exposure of rats to hydroxyurea in drinking water (~400–460 mg/kg bw/day for 70–90 days) reduced testis weight, affected Sertoli cell function, and induced histologic abnormalities in seminiferous tubules (Mecklenburg et al., 1975; Rich and De Kretser, 1977).

A multiple dose-level study in mice (Evenson and Jost, 1993) showed detrimental effects on testicular germ cell distribution beginning at hydroxyurea doses of 50 mg/kg bw/day i.p. for 5 days.

Pseudopregnant rats treated with hydroxyurea 500 mg/kg bw/day s.c. on pseudopregnancy Days 5–8 showed a decrease in weight of the endometrium and decreased endometrial protein and DNA content (Spencer et al., 2000), suggesting that hydroxyurea affected cell proliferation in the endometrium. There are no other relevant experimental animal data regarding potential female reproductive toxicity of hydroxyurea.

There are inadequate experimental animal studies to completely evaluate the effect of hydroxyurea exposure on fertility and male and female reproduction. The existing data show that hydroxyurea impairs spermatogenesis in laboratory animals. These data are assumed relevant to the assessment of hydroxyurea as a potential reproductive hazard in humans.

5.2 Summary of Human Exposure

Hydroxyurea is FDA-approved for adults with sickle cell disease and for the treatment of a number of malignancies, including chronic myelocytic leukemia. Off-label uses include treatment of other myeloproliferative disorders, psoriasis, HIV infection, and treatment of children with sickle cell disease. It is unknown how many adults and children are currently being treated with hydroxyurea and what the length of their exposure is. It is not known how many pregnant or nursing women are exposed to hydroxyurea, resulting in the exposure of the fetus or child. No information is available on possible occupational exposure to hydroxyurea.

No reliable information is available on the metabolism of hydroxyurea in humans. Indefinite treatment with hydroxyurea is recommended if the treatment is found to be effective and the patient shows no evidence of toxicity. Recommended starting doses of 10–20 mg/kg bw/day and maximum doses of 25–35 mg/kg bw/day have been used in adults and children with sickle cell disease (Ohene-Frempong and Smith-Whitley, 1997; NHBLI, 2002). Higher doses are recommended in the treatment of solid tumors. A reduction in dose is recommended in patients with impaired renal function. Hydroxyurea treatment is not recommended currently in pregnant women or lactating mothers. There are insufficient data on frequency of use and potential adverse effects of hydroxyurea in pregnant women and lactating mothers.

The most common adverse effect reported in patients taking hydroxyurea is suppression of the bone marrow, which most often results in neutropenia (Bristol-Myers-Squibb, 2004). Thrombocytopenia and anemia can also occur. Hydroxyurea therapy is associated with skin ulceration in patients with myeloproliferative disorders. When skin ulceration occurs in patients with sickle cell disease, it is unclear whether the ulceration is related to the underlying disease or the hydroxyurea.

In drug labels, hydroxyurea has been classified as an unequivocal genotoxicant. However, studies of genetic toxicity in humans are inconclusive as they are limited by lack of control data, small sample size and insufficient length of follow-up. Chronic exposure to hydroxyurea has been associated with hematologic malignancies. IARC (IARC, 2000), however, concluded that available data did not allow a conclusion on whether the occurrence of acute leukemia or myelodysplastic syndrome in patients treated with hydroxyurea for myeloproliferative disorders represented progression of the myeloproliferative disorder or an effect of treatment. There is insufficient long-term follow-up data to assess the hydroxyurea-associated risk of malignancy or other toxicities in children and adults with sickle cell disease.

5.3 Overall Conclusions

- **The Expert Panel has concern that hydroxyurea may increase the risk of congenital anomalies or abnormalities of fetal growth and postnatal development after exposure of pregnant women.** This conclusion is based on the animal data indicating that hydroxyurea produces congenital anomalies and abnormalities of fetal growth in multiple experimental species. Data in experimental animals show that exposure levels causing developmental toxicity produce blood concentrations similar to those achieved in patients on therapy. The Expert Panel recognizes that hydroxyurea is used to treat serious illnesses and that the decision to use hydroxyurea by a woman of reproductive age or by a pregnant woman is made by the patient and her clinician.[4]
- **The Expert Panel has minimal concern about the adverse effect of hydroxyurea on growth in children exposed to therapeutic doses of hydroxyurea at 5–15 years of age.** The growth studies were limited by lack of long-term follow-up. There are inadequate data on growth effects in infants and children younger than 5 years.
- **The Expert Panel has concern about the adverse effect of hydroxyurea on spermatogenesis in men receiving hydroxyurea at therapeutic doses.** This conclusion is based on experimental animal data in rats and mice showing decreased testis weight and sperm count. Dose levels that caused adverse effects in the experimental animal studies are expected to produce blood concentrations that are similar to those achieved in patients on therapy. There are no data on the fertility of experimental animals after treatment with hydroxyurea. The Expert Panel recognizes that hydroxyurea is used to treat serious illnesses and that the decision to use hydroxyurea by a man of reproductive age is made by the patient and his clinician.

5.4 Critical Data Needs

- Studies are needed on patient populations with diseases that are potentially treated with hydroxyurea. Subpopulations of particular interest include infants, children and adolescents, people in their reproductive years, and pregnant and lactating women. The establishment of a registry of all people with sickle cell disease that includes patients taking and not taking hydroxyurea is one way of meeting this data need.

[4]Because children and young adolescents may not have the maturity to make informed decisions about reproductive health-related matters, the Expert Panel recognizes that clinicians caring for these children will involve parents, guardians, or other adults in some of these decisions. The Expert Panel also recognizes that some states require involvement of parents in reproductive health-related decisions affecting minor children.

- Further pharmacokinetic studies (absorption, distribution, elimination, metabolism) in patients on hydroxyurea therapy (especially children, adolescents, and pregnant and lactating women) are needed.
- More research is needed on the potential developmental toxicity of hydroxyurea to the fetus and newborn after maternal hydroxyurea therapy during pregnancy and lactation. One way to meet the research needs is use of the sickle cell disease registry to study women of reproductive age for whom systematic follow-up of pregnancy outcomes occurs. Prospective studies should be conducted.
- The Expert Panel urges the continued support of research assessing growth and development in children with sickle cell disease younger than 5 years of age exposed to hydroxyurea therapy.
- Hydroxyurea can be used for long periods of time and adverse effects may have long latent periods. Studies are needed to evaluate the adverse effects of long periods of exposure and to assess outcomes that take many years to manifest after exposure. Studies on growth and development need to be expanded to include follow-up substantially longer than in existing studies.
- Studies to assess fertility and potential effects on both male and female reproduction in people exposed as infants, children, adolescents, and adults are needed.
- Experimental animal studies are needed to evaluate long term effects of prenatal and postnatal hydroxyurea exposures (separately and together) on postnatal development, including (but not limited to) developmental neurotoxicity, reproductive function, and carcinogenesis. Studies with a multi-generation design with an oral route of exposure would help meet this need.
- Additional studies are needed to understand the beneficial and toxic effects of hydroxyurea at a mechanistic level.

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Appendix II

APPENDIX III. PUBLIC COMMENTS

Public comments received during the NTP-CERHR evaluation of hydroxyurea are available on the CERHR website at *http://cerhr/chemicals/hydroxyurea/pubcomm-hydroxyurea.html.*