SUPPORT FOR CHEMICAL NOMINATION AND SELECTION PROCESS OF THE NATIONAL TOXICOLOGY PROGRAM

EXECUTIVE SUMMARY OF DATA

URETHANE

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Submitted to:

National Toxicology Program
National Institutes of Health
Building 31, Room 2B-55
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Submitted by:

Division of Toxicology
Center for Food Safety and Applied Nutrition
Food and Drug Administration

CONTENTS

NTP EXECUTIVE SUMMARY OF DATA

URETHANE (ETHYL CARBAMATE)

I. Chemical and Physical Information

A. Synonyms : Ethyl carbamate
Ethyl ester of carbamic acid
Ethylurethan
Ethyl urethan
Ethyl urethane
Urethan

B. CAS No. : 51-79-6

C. Molecular Formula : $C_3H_7NO_2$

D. Structural Formula : $H_2N - \overset{\overset{O}{\|}}{C} - OC_2H_5$

E. Molecular Weight : 89.09

F. Physical Properties :

1. Physical State : Colorless, almost odorless, columnar crystals or white, granular powder
2. Melting Point : 48-50°C
3. Boiling Point : 182-184°C
4. Specific Gravity : 1.1
5. Solubility : At 25°C, 1 gram dissolves in 0.5 ml water, 0.8 ml ethanol, 0.9 ml chloroform, 1.5 ml ether, 2.5 ml glycerol, and 32 ml olive oil (Merck Index, 1976).

G. Volatility : Sublimes readily at 103°C at 54 mm Hg pressure (Merck Index, 1976).

II. Production/Use/Exposure/Environmental/Regulatory Data

A. Production

As of 1978, urethane was produced by two companies in the U.S. - Kewanee Industries, Inc., a subsidiary of Millmaster Onyx Corp. in Berkley Heights, N.Y. and M and M Chems, Inc., Functional Plastics Division, Los Angeles, CA (EPA, 1979). The only estimate of production level available is greater than 1,000 pounds.

Although it reportedly can be made by the reaction of ethanol and urea under pressure and by warming urea nitrate with ethanol and sodium nitrite, the method used for commercial production is probably the reaction of ethyl chlorocarbonate (made from ethanol and phosgene) with ammonia (IARC, 1974).

The 1979 TSCA Inventory identifies five companies producing 6,105,000 million pounds of urethane, with some site limitation. Several companies now produce urethane solely for research. (NTP, 1985)

B. Use

Urethane had once been used as an active ingredient in drug products for the treatment of neoplastic diseases, as a hypnotic, as an adjunct to sulfonamide therapy, as a component (with quinine) of a sclerosing solution for varicose veins, and as a topical bactericide. It had also been used as an inactive component in liquid preparations as a solubilizer for an active ingredient. In 1976, FDA withdrew the approvals for all drug products containing urethane either as an active or inactive ingredient, because of the lack of safety (shown to be an animal carcinogen) and effectiveness (FR, 41:9523, 1976).

C. Occurrence

In 1971, it was discovered that urethane could be formed in beverages to which diethyl pyrocarbonate (an antimicrobial agent used as a preservative in beverages and wines) had been added. It was later shown to occur as a result of the reaction of ammonia and diethyl pyrocarbonate added at levels of 10 ug/l in certain beverages at pH below 4.0 (Lofroth and Gejvall, 1971). Therefore, in 1972, FDA banned the use of diethyl pyrocarbonate in beverages and wines.

In 1976, it was further reported that urethane could be formed naturally in a variety of fermented foods and beverages (e.g. wine, beer, bread, yogurt, ale, olives and Japanese sake) at concentrations ranging from 0.6 to 192 ug/l or kg (Ough, 1976). The occurrence of urethane in these products was attributed to the nonenzymatic reaction of either carbamyl phosphate or urea contained in fermented beverages and foods with alcohol via ethanolysis. However, the FDA has not been able to confirm the presence of urethane in these foods.

In November, 1985, the Liquor Control Board of Ontario, Canada, announced that relatively high levels of urethane, in excess of naturally occurring amounts, had been detected in

certain types of wines and other alcoholic beverages sold in Ontario. BATF and FDA have also detected urethane in similar products sold in the U.S.

D. Data Needed by FDA for Regulatory Action

As will be discussed later, in our analyses of the available data it became evident that although there is sufficient evidence of the carcinogenicity of urethane in experimental animals (IARC Monograph, 7, 111-140, 1974), the assessment of the magnitude of its carcinogenic risk is extremely difficult in view of the deficiencies in the available carcinogenicity data base. Most of the available carcinogenicity data were from studies which were not designed primarily as conventional carcinogenic bioassays, administering the test substance for the lifetime of the experimental animals. In addition, in most of these studies with urethane, a single high dose was commonly used and the numbers of animal used were small.

FDA may be constrained to set an interim action level based on the available inadequate data. However, in order to assure that a final action level provides adequate protection for the public, a conventional bioassay with good dose-response design suitable for risk assessment of urethane is urgently needed.

III. Toxicological Effects

A. Human Data

1. Acute: Urethane was used as an anesthetic and in the treatment of leukemia in the past. About 50% of all patients taking urethane orally had side effects such as nausea and vomiting. Less than 50% suffered from drowsiness. Gastroenteric hemorrhages have occurred after prolonged administration. Large doses (more than 3 grams/day orally) have made debilitated patients more prone to hepatitis or fatal hepatic necrosis. (EPA, 1979)

2. Epidemiological Evidence/Case Reports: No epidemiological studies or case reports were available.

3. Chemical Disposition: No information was found.

4. Biochemical Effects: No information was found.

5. Carcinogenicity/Chronic Toxicity: No information was found.

6. Teratogenicity and Reproductive Effects: No information was found.

B. Animal Data

1. Acute: The acute toxicity (LD_{50}) of urethane in mice and rats is presented in Table 1.

Table 1. LD_{50} Values of Urethane In Mice and Rats (EPA, 1979)

Species	Intraperitoneal	Subcutaneous	Oral	Intramuscular
Mouse	1200 mg/kg	2230 mg/kg	2700 mg/kg	--
Rat	1500 mg/kg	1800 mg/kg	--	1400 mg/kg

The primary effect of an acute exposure to urethane in animals is bone marrow depression. Focal degeneration of the brain, central nervous system depression and vomiting are sometimes produced. (EPA, 1979)

2. Chemical Disposition and Biochemical Effects: The metabolism of urethane in experimental animals has been reviewed by IARC (1974).

When urethane is administered to rats and pregnant mice, it is rapidly and evenly distributed throughout the body and is found in the body fluids of the rats and of the mouse foetuses. In mice, about 90% of the administered dose is excreted within 24 hours as CO_2 in the expired air, about 6% remains in the body and an approximately similar amount is excreted in the urine. Urethane injected i.p. in newborn mice is eliminated at only a tenth of the adult rate: in newborns 20% of the administered urethane is eliminated after 24 hours; in adults, however, 75% is eliminated within 6 hours. The rate of elimination of urethane increases slowly in newborn mice for the first 10 days after birth and sharply between the 15th-20th days. The longer retention time of urethane in newborn animal liver is attributed to the lack of an esterase which metabolizes urethane to CO_2, and which is active in adult liver microsomes.

In rats, rabbits and humans (patients with multiple myeloma treated with urethane in conjunction with an alkylating agent), the urinary metabolites are: urethane (0.5 - 1.7% of the administered dose), N-hydroxy urethane (0.02 - 0.15%), acetyl-N-hydroxy urethane (0.1 - 0.6%),

ethyl mercapturic acid (0.1 - 0.2%) and N-acetyl-S-ethoxy carbonylcysteine (0.9 - 2.1%). The urinary metabolites of N-hydroxy urethane are qualitatively similar but quantitatively different. N-hydroxy urethane is also excreted as glucuronide, resistant to hydrolysis by mineral acids and by beta-glucuronidase.

N-oxidation of urethane to form N-hydroxy urethane, N-hydroxy esters or free radicals, resulting from abstraction of a hydrogen atom attached to the amido nitrogen, leads to biologically active ethoxycarbonylation and/or, by the loss of CO_2, to ethylating species which produce derivatives of cytosine and sulphur-containing amino acids. Such reactions occur with tissue sulphhydryl groups and S-ethyl, and S-ethoxycarbonyl derivatives of N-acetylcysteine are mainly excreted. Inorganic one-electron oxidation of alkyl-N-hydroxycarbamates leads to alkoxy carbonylating species and to the formation of the corresponding alkyl carbamates. The metabolic "reduction" of N-hydroxy urethane which is commonly observed in vivo may be mediated by such an oxidative mechanism.

Recently, it was reported that following treatment of mice and rats with [ethyl-1-^{14}C]-ethyl carbamate, the main radioactive DNA adduct was identified as 7-(2-oxoethyl) guanine by cochromatography with the authentic marker in several separation systems. Administration of vinyl carbamate led to about 100 times as much 7-(2-oxoethyl, guanine (on a molar basis) as did ethyl carbamate strongly supports the existence of the metabolic activation pathway, such as ethyl carbamate -- vinyl carbamate -- epoxyethyl carbamate (Allen, et al., 1982; Scherer, et al., 1986).

3. Review of the Available and Pertinent Carcinogenicity Data: The literature pertaining to urethane carcinogenicity is too extensive and numerous to be included fully in this summary. Most of the studies were conducted with unusual protocols and do not meet the current conventional bioassay protocol. Only those selected studies which represented the best available studies (judging from their close compliance to the current testing protocol) are summarized here.

The subject of the carcinogenicity of urethane had been reviewed previously (Mirvish, 1968; IARC, 1974; EPA, 1979).

(i) Schmahl, et al. (1977) study in rats and mice:

Rat Study

Groups of SPF Sprague-Dawley rats (40 rats/sex/group) were given urethane in the drinking water at dose levels of 0 (control), 100, 500, 2500 and 12500 ug/kg/day during their lifetime. The experiment for the three higher dose groups (12500, 2500 and 500 ug/kg/day) were terminated after 670 days, while the lower dose group (100 ug/kg/day) was ended after 730 days, and the control group lasted for 680 days. About 8% of the total effective number of animals were still alive and were sacrificed at the end of the experiment. All animals were autopsied. Those organs which macroscopically showed signs of alteration were examined histologically.

The investigators reported that about 50% of the total effective animal number were lost between 350 and 450 days after the beginning of the experiment, probably due to an unspecified viral infection. Nevertheless, the investigators reported that the final yield of animals bearing malignant tumors increased fairly steadily with increasing dose, beginning with 500 ug/kg/day. The proportion of animals with benign tumors was increased at the two higher dose levels. At the highest dosage, 9 of the 15 malignant tumors were mammary carcinomas, 2 were vascular and 2 were neurogenic tumors. Seven of the 8 benign tumors in this group were mammary fibroadenomas.

In the report, there is no breakdown of tumor-type seen in each organ and because only macroscopic signs of alterations were examined histopathologically, the exact tumor incidence in each organ could not be determined from the limited information provided. After contacting the principal investigator, we were informed that no further information is available to help us clarify the questions we raised. Consequently, this study is not suitable for the purpose of risk assessment.

Mouse study

Groups of SPF NMRI mice (40 mice/sex/group) were given urethane in the drinking water at dose levels of 0 (control), 100, 500, 2500 and 12500 ug/kg/day for their lifetime (the highest dose group - 660 days; 3 lower dose groups - 730 days; control group - 760 days). Approximately 13% of the starting number of animals were still alive and were sacrificed at the end of the experiment. Those organs showing gross abnormality were examined histopathologically.

It was reported that the final yield of animals with malignant tumors increased steadily with increasing dose, beginning with 100 ug/kg/day; the proportion of animals with benign tumors increased with dose from 500 ug/kg/day and up. Those tumors which showed a clear dose-dependence were tumors of the lung (mainly adenomas and adenocarcinomas), malignant mammary tumors, and hemangioendotheliomas (mainly in the liver), but no incidences were provided. Again, this mouse study could not be appropriately used for risk assessment, because it suffers the same defects as those discussed in the rat study.

The following studies involved only one group of animals exposed to urethane alone.

(ii) Toth and Boreisha (1969) study on Syrian golden hamster:

This study was carried out primarily to determine whether isonicotinic acid hydrazide (INH) enhanced urethane-induced lung tumorigenesis. Among the four experimental groups in this study, only one group (Group 1) dealt with urethane alone and it will be considered here along with the corresponding control group. In the urethane-treated group, 52 male and 48 female five-week old Syrian golden hamsters were administered urethane in their drinking water at concentration of 0.1% w/v for 140 weeks. The control group, consisting of 100 males and 100 females, was given only tap-water for the same period. The average urethane intake in the treated group was estimated to be 15.1 mg/day/animal and 15.4 mg/day/animal for males and females, respectively. Complete necropsies were performed on all animals and all organs were examined grossly. Histological examinations were performed on the liver, kidney, spleen, and at least 4 lobes of the lungs of each hamster and on organs which showed gross pathological changes.

The urethane-treated animals appear to have a significant decrease in the survival rate as compared to the control. The organs/tissues which were reported to have increased tumor incidences include: papillomas and carcinomas of the forestomach (treated - Male, 52/52, 100%; Females, 42/48, 88%; Control - Male, 6/100, 6%; Female, 2/100, 2%); dermal melanocytomas (treated-Male, 26/52, 50%; Female, 25/48, 52%; control - Male, 1/100, 1%; Female, 0/100, 0%) and adenomatous polyps of cecum (treated - Male, 4/52, 7%; Female, 7/48, 14%; control - Male, 0/100, 0%; Female, 0/100, 0%).

It is clear that this study was carried out to test for the carcinogenic effects of urethane at a single chronic dose level with very limited microscopic examination and has limited value in dose-response analysis and risk assessment.

(iii) Tomatis, et al. (1972) study on CF-1 mice:

The main purpose of this study was to test the chronic toxicity/carcinogenicity of DDT, and urethane given at a single concentration of 0.01% (20 mg/kg/day) in the drinking water ad libitum served as a positive control. Each experimental group consisted of 60 males and 60 females. The treatment period lasted for the entire-life span of the animals for two consecutive generations (140 weeks for the parental generation and 130 weeks for the F_1 generation). When 9 to 10 weeks old, 20 females (parent generation, P) from each group were mated with males from their respective treatment groups to obtain the first generation (F_1). All mice were autopsied and the lung, heart, thymus, liver, kidney, spleen, gonads, and brain together with all organs showing gross abnormalities were histologically examined.

It was reported that there was no difference in survival rates between urethane-treated and the control groups up to 90 weeks, at which time approximately 50% of the animals in each of these groups were alive. After 90 weeks, the urethane-treated group exhibited higher mortality than the controls. Urethane treatment induced primarily lung tumors and osteomas in the P and F_1 males and females. The tumors incidences in the treated group were as follows:

	Lung Tumors		Osteomas	
	M (%)	F (%)	M (%)	F (%)
P - Generation				
Urethane-treated	40/48 (83)	28/40 (70)	N.R.	7/40 (18)
Control	23/55 (42)	13/56 (23)	8/55 (15)	15/56 (27)
F_1 - Generation				
Urethane-treated	56/61 (92)	31/38 (82)	8/61 (13)	9/38 (24)
Control	18/58 (31)	22/55 (40)	3/58 (5)	9/55 (16)

As noted, these studies tested only one single dose level of urethane for only 90 weeks, and microscopic

examinations were limited to a few organs, therefore, limiting their utilization in risk assessment.

(iv) Van Esch and Kroes (1972) study on mice and hamsters:

In the study of the chronic toxicity of chloropropham in Swiss mice, an 0.1% urethane in the diet was included to serve as a positive control. The untreated control group was given standard diet only. There were 25 males and 25 females in each group. The study lasted for 116 weeks and after which all surviving animals were killed and examined macro- and microscopically. Tissues from the liver, kidney, heart, lungs, spleen, pancreas, adrenals, gastrointestinal tract, urinary bladder, prostate, testes, ovaries and uterus were examined histologically. The total numbers of mice examined microscopically were reported to be 49 for the untreated control and 48 for the 0.1% urethane-treated group, but they were not divided by sex.

It was reported that both male and female mice given 0.1% urethane died earlier than untreated controls. The average life span of urethane-treated animals was 40 weeks as compared to 75 weeks for the controls, but no detailed survival rates were given. The only significant increase in tumor incidence in the urethane-treated group is multiple (mostly adenomas) lung tumors; they were present in 46 of the 48 mice (96%) given urethane as compared to 10 of the 49 (21%) in the control. In addition, one animal in the urethane group also had lung adenocarcinoma. The average numbers of lung tumors in the affected animals of the control and urethane-treated groups were 2.5 (1 to 8 tumors) and more than 20 tumors, respectively.

Again, this study tested only a single high dose of urethane with few animals per group. It has limited value in conducting risk assessment.

(v) Innes, et al. (1969) study on mice:

In this study to test the tumorigenicity of 110 selected pesticides and industrial chemicals by continuous oral administration of MTD doses of the test compounds to both sexes of two hybrid mouse strains: C57BL/6 x C3H/Anf and C57BL/6 x AKR; urethane was included to serve as positive control (24 mice for each sex and hybrid were used).

Urethane at MTD dose of 158 mg/kg was given by gavage between days 7 and 28 after birth. Subsequently, animals were fed a diet containing 600 ppm urethane ad lib. for 18 months. Histologic examination of major organs and all grossly visible lesions was performed. The urethane-treated animals were compared with all negative control groups with respect to the incidence of hepatomas, pulmonary tumors, lymphomas, and total number of mice with tumors.

Pulmonary tumors and hepatomas were found to be increased in the urethane-treated mice as follows:

Pulmonary tumor

Mouse strain	Control		Urethane-treated	
	M	F	M	F
C57BL/6xC3H/Anf	5/79(6%)	3/87(3%)	6/20(30%)	6/23(26%)
C57BL/6 x ARK	10/90(11%)	3/82(4%)	15/22(68%)	17/19(89%)

Hepatoma

Mouse strain	Control		Ureathane-treated	
	M	F	M	F
C57BL/6 x C3H/Anf	8/79(10%)	0/87(0%)	8/20(40%)	12/23(62%)
C57BL/6 x AKR	5/90(6%)	1/82(1%)	14/22(62%)	5/19(26%)

Although no data were presented, the investigators indicated that a substantial number of adenomas of the harderian gland was observed in urethane-treated animals.

Because this study tested only one single dose, it could not be utilized for a meaningful risk assessment.

4. Other relevant toxicological data:

(i) Two-stage tumor initiation-promotion.

It had been reported that urethane is an initiator for mouse skin carcinogenesis promoted by phorbol esters (Slaga, 1983) and for mouse lung carcinogenesis promoted by BHT (Witchi, 1977; Witchi, 1981).

(ii) Effects of partial hepatectomy on urethane carcinogenicity.

Several carcinogenicity data have suggested that, although urethane is capable of producing cancer at various sites,

it does not in general induce liver tumors in adult mice and rats, but does so when given as a single high dose application at birth or soon afterward (Toth, 1970). However, it had been demonstrated that prior partial hepatectomy can substantially increase liver tumor yield in several strains of adult mice when urethane is administered following the operation (Hollander and Bentvelzen, 1968; Lane, et al., 1970; Chernozemski and Warwick, 1970).

5. Teratogenic Effects: As indicated by its transplacental carcinogenic activity, urethane readily traverses the placenta and can be found in the fetus. Teratogenic effects observed are skeletal defects and cleft palate observed after administration of 1.5 gm/kg/day given to mice from the ninth to the twelth day of gestation, neural tube closure defects seen after an injection of 15 mg of urethane into mice on the seventh day of gestation, and eye development defects in rats exposed in utero. An in vitro experiment showed digital malformations developed in both hindlimbs and forelimbs from days 12 and 13 mouse embryos after being cultured with urethane. Both polydactyly and syndactyly were found. These same malformations were seen when urethane was given by subcutaneous injection to pregnant mice on day 11. Malformations of external appearance (digital malformations, tail anomalies and cleft palate) were found when the injection was given on days 9-12 of gestation and internal malformations (anomalies of the lung and liver) were seen after administration on day 8 or 9. Administration after day 13 caused the development of lung tumors in the offspring (EPA, 1979).

C. Mutagenicity

Results of mutagenicity experiments with urethane are mixed. Gene mutations are not induced in Salmonella, with or without S9 microsomal activation (McCann, et al., 1975; Bruce and Heddle, 1979), but dose-related induction of sex-linked recessive lethals has been reported for Drosophila (Nomura, 1979). In the mouse spot test, Tutikawa and Harada (1972) found no induction of recessive spots in 3 different crosses, but Nomura (1983) reported a significant increase in such spots. Chromosome aberrations have been found to be induced in rodent bone marrow, analyzed either for micronucleus formation (Bruse and Heddle, 1979) or by cytogenetic and/or banded karyotype analysis (Dean, 1969; Sugiyama et al., 1981). In Drosophila germ cells, on the other hand, urethane failed to produce a significant increase in translocations (Nomura, 1979).

In mammalian germ cells, urethane does not produce spermhead abnormalities when differentiating spermatogonia of the mouse are exposed (Wyrobek and Bruce, 1975; Bruce and Heddle, 1979; Topham, 1980). On the other hand, urethane inhibits testicular DNA synthesis (Seiler, 1977); and sister-chromatid exchange is induced in spermatogonia in a dose-related fashion (Roberts and Allen, 1980), although with lower frequency than produced by similar doses in somatic tissues (Csukas, et al., 1979; Roberts and Allen, 1980; Csukas, et al., 1981; Cheng, et al., 1981; Allen, et al., 1982; Conner and Cheng, 1983; Dragani et al., 1983; Sharief, et al., 1984; Neff et al., 1985; Allen, et al., 1986). But, it has been shown recently that urethane failed to induce germ-cell mutation as studied by specific-locus test (Russell, et al., 1987).

With regard to genetic effects transmitted to the next generation of mice, the evidence is mixed. Nomura reported the induction of dominant lethals in 1975 (Nomura, 1975), but later reported that there was no significant increase of dominant lethals from urethane treatment at any stage (Nomura, 1982). Negative dominant-lethal results have also been obtained by a number of investigators (Bateman, 1967; Epstein, et al., 1972; Russell, et al., 1987). On the other hand, Nomura's laboratory had reported significant increases in morphological malformations and, most intriguingly, of tumors (almost exclusively lung adenomas) in the progeny of mice exposed to urethane either as fetuses (Nomura, 1975) or as adults (Nomura, 1982).

D. Structure-activity relationship

A structural analog of urethane, methyl carbamate, has been reported recently by NTP (1986) to be carcinogenic for male and female F-344 rats, inducing hepatocellular neoplastic nodules and hepatocellular carcinomas, when administered in distilled water by gavage, 5 days per week for 103 weeks, at doses of 100 and 200 mg/kg.

IV. Nomination Source

A. Source: Division of Toxicology, Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration.

B. Recommendation: Chronic toxicity and carcinogenicity bioassay with a good dose-response design suitable for risk assessment of urethane is recommended.

C. Rationale/Remarks:

In this summary we have briefly reviewed the pertinent data of urethane. It has been shown to be a multipotential carcinogen. Although the carcinogenic target of urethane has been shown to be primarily the lung in various strains of mice, various other types of tumors have been induced in several species. The carcinogenicity of urethane appears to be dependent on age, sex, species and strain.

As noted above, most of the available data are from studies which were not designed primarily as conventional carcinogenic bioassays. In most of the chronic studies with urethane, a single high dose was generally used, with a small number of animals.

Among the five "best" available studies reviewed herein, the Schmahl study appeared to be the most suitable for risk assessment of urethane. The study was designed for dose response (with 4 different doses) during a lifetime drinking water administration. However, there are several problems in utilizing the data derived from this study: (1) Loss of a large number of animals in the early period of the study due to unknown infection had been noted; (2) There was no breakdown of tumor type seen in each organ; and (3) Only macroscopic signs of alterations were examined histologically, thus limiting the determination of exact tumor incidence in each organ needed for risk assessment.

Although the other four studies were also essentially conducted for the lifetime of the animals, there was only one single dose administered in all four studies, limiting the usefulness of these data for risk assessment. Therefore, it is evident that a meaningful assessment of the magnitude of the carcinogenic risk of urethane is not possible utilizing the available carcinogenicity data base. Although FDA may set an interim action level based on the available inadequate data, but in order to assure that the final action level provides adequate protection for the public, FDA urgently needs a conventional bioassay with a good dose-response design suitable for risk assessment of urethane.

D. Priority: The highest priority is recommended because of the possible widespread exposure of the general population from alcoholic beverages.

E. Date of Nomination: September, 1987

V. Information Sources

This report was prepared by Chiu S. Lin, Ph.D. of the Division of Toxicology, CFSAN, FDA.

The information resources used in preparing this report include the on-line database searches from National Library of Medicine (MEDLARS), up to 1986, general reference materials, agency reports (IARC, 1974; EPA, 1979; NTP, 1985) and journal articles.

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