NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for DANTHRON

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TABLE OF CONTENTS

NTP Report on Carcinogens Listing for Danthron

Carcinogenicity

Danthron is *reasonably anticipated to be a human carcinogen* based on evidence in experimental animals (reviewed in IARC, 1990). When administered in the diet to male rats, danthron induced adenomas and adenocarcinomas of the colon and adenomas of the cecum. When administered in the diet to male mice, danthron caused an increase in the incidence of hepatocellular carcinomas.

There are no adequate data available to evaluate the carcinogenicity of danthron in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Danthron has been evaluated in studies for its ability to enhance the expression of tumors induced by other chemicals. When danthron was administered in the feed to mice that also received 1,2-dimethylhydrazine, the incidence and multiplicity of adenomas of the colon and liver were significantly increased (Sugie et al., 1994). When evaluated in skin painting studies in mice given 7,12-dimethylbenz[*a*]anthracene, or in rats given 1,2-dimethylhydrazine, danthron gave negative results (IARC, 1990). When administered in the diet without other chemicals, danthron caused a large increase in the incidence of a preneoplastic lesion, adenomatous polyploid hyperplasia of the cecum and colon in male mice. Danthron has been found to induce genetic damage in a limited number of prokaryotic, lower eukaryotic, and mammalian *in vitro* test systems.

No data are available that would suggest that the mechanisms thought to account for tumor induction by danthron in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded; or

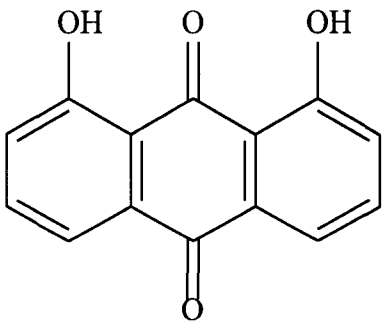
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgement, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

Danthron
[117-10-2]



1.1 Chemical Identification

Danthron ($C_{14}H_8O_4$, mol. wt. = 240.22) is also called:

9,10-Anthracenedione, 1,8-dihydroxy- (9CI)	Dorbanex
Anthraquinone, 1,8-dihydroxy- (8CI)	Duolax
Altan	Istin
Antrapurol	Istizin
Chrysazin	Laxanorm
component of Dorbantyl	Laxanthreen
component of Doxan	Laxipur
component of Doxidan	Laxipurin
component of Modane	LTAN
Criasazin	Modane
Danivac	Neokutin S
Dantron	Pastomin
Dantrona	Prugol
Dantrone	Regulin
Dantronum	Roydan
Diaquone	Scatron D
1,8-Dihydroxy-9,10-anthracenedione	Solven
1,8-Dihydroxyanthraquinone	1,4,5,8-Tetroxyanthraquinone
1,8-Dihydroxy-9,10-anthraquinone	Unilax
Dionone	USAF nd-59
Dorbane	Zwitsalax

1.2 Physical-Chemical Properties

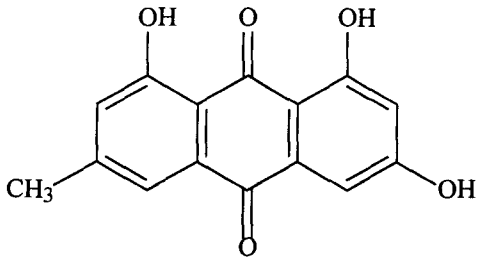
Property	Information	Reference(s)
Color	Orange	Budavari (1996)
Physical State	Needle crystals	Budavari (1996)
Melting Point, °C	193-197	Budavari (1996)
Solubility:		
Water at 25 °C	6.5 μmol/L	Budavari (1996)
	Very slightly soluble in aqueous alkali hydroxides	Enviro Control (1981) and Weast (1985); both cited by IARC (1990)
Organic Solvents	Soluble in glacial acetic acid, acetone, chloroform, diethyl ether, and ethanol	Enviro Control (1981) and Weast (1985); both cited by IARC (1990); Budavari (1996)

1.3 Identification of Structural Analogues and Metabolites

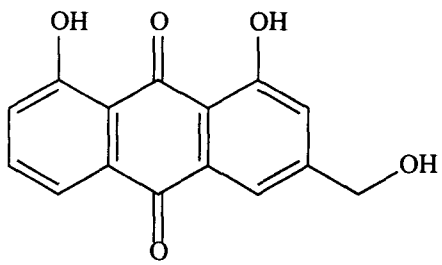
Structural analogues and metabolites discussed in this report include the following:

1,3,8-Trihydroxy-6-methyl-9,10-anthracenedione (Emodin)
1,8-Dihydroxy-3-hydroxy-9,10-anthracenedione (Aloe-Emodin)

Emodin is practically insoluble in water. It is soluble in alcohol, aqueous alkali hydroxide solutions with cherry red color, and Na_2CO_3 and NH_3 solutions. Aloe-Emodin is freely soluble in hot alcohol, ether, benzene with yellow color, ammonia, water, and sulfuric acid with crimson color (Budavari, 1996).



Emodin



Aloe-Emodin

1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Studies, and 7.0 References) and two appendixes. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1.

2.0 HUMAN EXPOSURE

2.1 Use

Danthron has been widely used since the beginning of this century as a laxative (IARC, 1990). FDA ordered its withdrawal from the market for this purpose in 1987 (58 FR 46589, 1993). It has also been used to a lesser extent as an intermediate in the manufacture of dyes and forms lakes with calcium, barium, and lead (Kirk-Othmer V.11, 1980).

2.2 Production

Danthron is synthesized in Germany, India, Japan, Poland, the UK, and the United States (IARC, 1990). The SRI Directory of Chemical Producers reported that one U.S. company produced an unknown quantity of danthron in 1992 (SRI, 1992). The TSCA inventory for U.S. plants and producers in 1977 listed 8 plants that produced or imported danthron. Three of the 8 were known manufacturers, 3 were known importers, and it was not known whether the other 2 were importers or manufacturers. The order of magnitude of the production volume was given for only one known manufacturer (100,000 to 1,000,000 lb/yr). One producer or importer handled 1,000 to 10,000 lb/yr. Two of the 3 known producers did not ship danthron out of the plant; i.e., its production and use were site-limited (TSCAPP, 1983 update). No data on imports or exports of danthron were available. In 1987, about 40 small manufacturers of danthron-containing pharmaceuticals were directed by FDA to withdraw their products from the market (Diogenes, 1976-1996). Chem Sources (1996) identified 17 U.S. suppliers of danthron.

2.3 Environmental Exposure

2.3.1 Environmental Occurrence

Danthron occurs naturally in several species of plants and insects. It has been isolated from dried leaves and stems of *Xyris semifuscata* harvested in Madagascar and is the basic structure of the aglycones of naturally occurring laxative glycosides. The compound has been identified in larvae of the elm-leaf beetle *Pyrrhalta luteola*. The presence of a mixture of anthraquinones and anthrones was suggested to be a means of protection from predators, and these compounds appear to be biosynthesized by the insect (IARC, 1990).

2.3.2 Consumer Products

Shortly before its withdrawal from the laxative market in 1987, danthron was available from 9 companies in 14 over-the-counter (OTC) products with the following trade names: Danivac, Doctate-P, Dorbane, Dorbantyl, Dorbantyl Forte, Doxan, Doxidan, Magcyl, Modane, Tonelax, West-Ward Dioctyl with Danthron, and Valax. Tablet formulations contained 37.5, 50, or 75 mg danthron; capsule formulations, 25, 40, or 50 mg; and a liquid formulation, 37.5 mg/5 mL (5 mL = 1 teaspoonful) (CTCP, 1985). A liquid product named Dorbanex, which is mentioned in Section 3.0, contained danthron with poloxalkol. In one case report, Dorbanex was administered orally at a dose of 5 mL/day (Patel et al., 1989). In another study, Dorbanex was reported to contain 25 mg danthron in 5 mL (Blair et al., 1977).

2.3.3 Occupational Exposures

The primary route of potential human exposure to danthron is oral administration. Potential exposure of health professionals may occur during the preparation and administration of the compound (the laxative was given to pregnant women in hospitals to induce labor). Potential occupational exposure may also occur for workers involved in the formulation and packaging of the pharmaceutical. The National Occupational Exposure Survey (1981-1983) indicated that 357 workers, including 187 women, were potentially exposed to danthron (NIOSH, 1984). This estimate was derived from observations of the use of the actual compound (47% of total observations) and tradename products (54%). The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 3,120 workers were potentially exposed to danthron in the workplace (NIOSH, 1976).

2.4 Regulations

In 1987, the FDA published a letter and a press release to recall all danthron-containing drug products by about 40 small manufacturers. Larger manufacturers had voluntarily halted production before the advisement. Both publications are in OTC Vol. 090TFM2, Docket 78N-036L (58 FR 46589, 1993; Diogenes, 1976-1996).

3.0 HUMAN STUDIES

3.1 Case Reports

A small bowel sarcoma was detected in an 18-year-old girl who had been treated with the laxative Dorbanex* (danthron with poloxalkol; 5 mL/day orally) from the age 14 months to the age of 5 or 6 years. Although regular treatment was discontinued at age 5 or 6 years, intermittent use continued throughout the rest of her life (Patel et al., 1989).

3.2 Cohort Studies

No cohort studies that evaluated the carcinogenicity of danthron in humans were found.

4.0 MAMMALIAN CARCINOGENICITY

Experimental details for the studies described in this section are presented in Table 4-1.

Summary: There is "sufficient evidence" for the carcinogenicity of danthron in experimental animals (IARC, 1990). In mice, the incidence of hepatocellular carcinoma was increased in males (females not evaluated) administered 2000 ppm danthron in the diet for up to 540 days. In rats, the incidence of intestinal tumors (adenoma and adenocarcinoma of the colon and adenoma of the cecum) was significantly increased in males (females not evaluated) administered 10,000 ppm danthron in the diet for 16 months.

4.1. Mice

The incidence of hepatocellular carcinoma was significantly increased in male C3H/HeN mice (females not evaluated) administered 2000 ppm danthron in the diet, beginning at 8 weeks of age, for up to 540 days. All carcinomas in the danthron-treated mice, however, occurred in animals that also had adenomas, so the total number of liver-tumor-bearing mice was similar in dosed and control groups. The incidence of hepatocellular adenoma did not differ significantly between danthron-treated mice and controls fed basal diet alone (Mori et al., 1986).

4.2 Rats

The incidence of intestinal tumors (adenoma and adenocarcinoma of the colon and adenoma of the cecum) was significantly increased in male ACI rats (females not evaluated) administered 10,000 ppm danthron in the diet, beginning at 8 weeks of age, for 16 months (Mori et al., 1985). In a review by IARC (1990), but not in the report by Mori et al. (1985), it was noted that the incidence of colon tumors, but not of cecal tumors, was significantly increased in the danthron-treated rats.

*The Dorbanex product used by Blair et al. (1977) contained 25 mg danthron in 5 mL.

Table 4-1. Mammalian Carcinogenicity of Danthron

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice – *Oral Administration*							
8-wk-old C3H/HeN mouse	20M	20M (basal diet alone)	danthron, no impurities detected on thin-layer chromatography	2000 ppm in diet	up to 540 days	Mice were killed when moribund or at the end of the treatment period. At necropsy, special attention was given to the livers of the mice. The mean survival time and mean body weight of danthron-treated mice did not differ significantly from those of controls. **Liver:** Positive (for carcinoma) The incidence of hepatocellular adenoma did not differ significantly between danthron-treated and control mice (9/17 vs. 5/19 controls). The incidence of hepatocellular carcinoma was significantly increased in danthron-treated mice (4/17 vs. 0/19 controls [$p < 0.05$, Fisher's exact test]). The 4 carcinomas in the danthron-treated mice, however, occurred in animals that also had adenomas, so the total number of liver-tumor-bearing mice is similar in dosed and control groups.	Mori et al. (1986)
Rats – *Oral Administration*							
8-wk-old ACI rat	18M	15M (basal diet alone)	Danthron, "pure"	10,000 ppm in diet	16 mo	Rats were killed when moribund or at the end of the treatment period. **Intestinal Tract:** Positive (for adenoma and adenocarcinoma in colon) The incidences of intestinal tumors (3-adenoma and 4 adenocarcinoma of the colon and adenoma of the cecum) was significantly increased in danthron-treated rats (7/12 vs. 0/14 controls [$p < 0.01$, statistical test not specified]). In the IARC review, p was given as < 0.02, statistical test not specified. IARC noted that the incidence of cecal adenoma was not significantly increased in danthron-treated rats (2/12 vs. 0/14 controls).	Mori et al. (1986); IARC (1990)

5.0 GENOTOXICITY

Studies of the genotoxic effects of danthron are summarized in Table 5-1.

Summary: Danthron was found to be genotoxic in a limited number of prokaryotic, lower eukaryotic, and mammalian *in vitro* test systems [see Genetic Activity Profile, Figure 5-1 (data limited to IARC, 1990)]. Danthron induced gene mutations in *Salmonella typhimurium* and *Saccharamyces cerevisiae;* chromosomal aberrations in human peripheral blood lymphocytes; unscheduled DNA synthesis (UDS) in mouse and rat hepatocytes. It did not inhibit intercellular communication in either Chinese hamster V79 cells or human fibroblasts, induce cell transformation in C3H/M2 mouse fibroblasts, nor induce micronuclei in rodent bone marrow polychromatic erythrocytes or in mouse peripheral erythrocytes. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*.

Information for studies reviewed in IARC was often limited to qualitative data with information on study design, doses tested, chemical purity, etc., generally not provided. In addition, for simplicity, multiple citations in IARC for the same genetic toxicity endpoint and test system were discussed as a group rather than cited individually.

5.1 Noneukaryotic Systems

Five papers cited by IARC (1990) reported the effect of danthron on gene mutations in prokaryotic systems. Danthron induced gene mutations in *S. typhimurium* strain TA1537 in both the presence and absence of exogenous metabolic activation (Brown and Brown, 1976; Liberman et al., 1982) and in strains TA2637 (Tikkanen et al., 1983), TA102 (Levin et al., 1984), and TA104 (Chesis et al., 1984) (oxidative mutant sensitive strains) in the presence of S9 activation only [LED = 2.0 μg/plate (0.008 μmol/plate)]. Strains TA100, TA98, TA1535, and TA1538 gave negative responses both with and without S9 [HID = 2000 μg/plate (8.3 μmol/ plate)](Brown and Brown, 1976; Liberman et al., 1982; Tikkanen et al., 1983).

5.2 Lower Eukaryotic Systems

Zetterberg and Swanbeck (1971; cited by IARC, 1990) found that danthron induced respiration-deficient mutations in the yeast *S. cerevisiae*. The strain and dose levels were not provided in the review.

5.3 Mammalian Systems *In Vitro*

5.3.1 DNA Damage

Mori et al. (1984) reported that 20 and 200 μM danthron for 20 hours induced UDS in mouse primary hepatocytes [LED = 4.8 μg/mL (20 μM)]. Mori et al. (1984) and Kawai et al. (1986; cited by IARC, 1990) reported that danthron also induced UDS in rat primary hepatocytes [LED = 4.8 μg/mL (20 μM)]. Probst et al. (1981; cited by IARC, 1990), however, found that danthron did not induce UDS in rat hepatocytes [HID = 120 μg/mL (500 μM)].

5.3.2 Chromosomal Damage

Carballo et al. (1981; cited by IARC, 1990) stated that danthron induced chromosomal aberrations in human peripheral blood lymphocytes in the absence of exogenous metabolic activation [LED = 10 μg/mL (42 μM)].

5.3.3 Cell Transformation

Westendorf et al. (1990) reported that danthron did not induce morphological transformation in mouse C3H/M2 fibroblasts tested from 3.0 to 100 μg/mL (1.3 to 417 μM) in the absence of metabolic activation [HID = 30 μg/mL (13 μM)].

5.3.4 Intercellular Communication

Zeilmaker and Yamasaki (1986; cited by IARC, 1990) concluded that danthron did not inhibit gap-junction intercellular communication in Chinese hamster lung V79 cells [HID = 3.0 μg/mL (13 μM)]. Si et al. (1988; cited by IARC, 1990) also reported that danthron failed to inhibit gap-junction intercellular communication in human fibroblasts [HID = 2.4 μg/mL (10 μM)].

5.4 Mammalian Systems *In Vivo*

NTP (1995) reported that danthron did not induce micronuclei in rodent bone marrow polychromatic erythrocytes or in mouse peripheral blood erythrocytes (species, doses, and route of administration were not provided).

Table 5-1. Summary of Danthron Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1 Noneukaryotic Systems							
Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100	*his* gene mutations	+/-	n.p.	n.g.	positive/ positive	Positive response in strain TA1537 only, both with and without S9 [LED = 2.0 µg/plate (0.008 µmol/plate)]. All other strains were negative- HID = 2000 µg/plate (8.3 µmol/plate).	Brown and Brown (1976); Liberman et al. (1982); Tikkanen et al. (1983); all cited by IARC (1990)
S. typhimurium oxidative mutant sensitive strains TA2637, TA102, TA104	*his* gene mutations	+/-	n.p.	n.g.	positive/ negative	Positive in all three strains only in the presence of S9. LED = 2.0 µg/plate (0.008 µmol/plate)	Tikkanen et al. (1983); Chesis et al. (1984); Levin et al. (1984); all cited by IARC (1990)
5.2 Lower Eukaryotic Systems							
Saccharomyces cerevisiae	respiration-deficient mutants	-	n.p.	n.g.	positive	Strain and dose levels were not provided.	Zetterberg and Swanbeck (1971; cited by IARC, 1990)
5.3 Mammalian Systems *In Vitro*							
5.3.1 DNA Damage							
primary mouse hepatocytes	unscheduled DNA synthesis (UDS)	NA	n.p.	4.8 to 48 µg/mL (20 and 200 µM) for 20 hours	positive	LED = 4.8 µg/mL (20 µM)	Mori et al. (1984)
primary rat hepatocytes	UDS	NA	n.p.	n.g.	positive	Positive responses were observed in two studies [LED = 4.8 µg/mL (20 µM)] and negative in one [HID = 120 µg/mL (500 µM)]	Kawai et al. (1986); Probst et al. (1981); both cited by IARC (1990); Mori et al. (1984)
5.3.2 Chromosomal Damage							
human peripheral blood lymphocytes	chromosome aberrations	-	n.p.	n.g.	positive	Dose levels and exposure time were not provided. LED = 10 µg/mL (42 µM)	Carballo et al. (1981; cited by IARC, 1990)
5.3.3 Cell Transformation							
C3H/M2 mouse fibroblasts	morphological transformation	NA	n.p.	3.0 to 100 µg/mL (1.3 to 417 µM), exposure time not provided.	negative	HID = 30 µg/mL (13 µM). The highest dose, 100 µg/mL (417 µM), precipitated out of the culture medium and caused complete toxicity.	Westendorf et al. (1990)

Table 5-1. Summary of Danthron Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.3.4 Intercellular Communication							
Chinese hamster lung V79 cells	inhibition of gap junction intercellular communication	-	n.p.	n.g.	negative	HID = 3.0 µg/mL (13 µM)	Zeilmaker and Yamasaki (1986; cited by IARC, 1990)
human fibroblasts (tissue source not provided)	inhibition of gap junction intercellular communication	-	n.p.	n.g.	negative	HID = 2.4 µg/mL (10 µM)	Si et al. (1988; cited by IARC, 1990)
5.4 Mammalian Systems *In Vivo*							
rodent (species not provided) bone marrow and mouse peripheral blood polychromatic erythrocytes	micronuclei induction	NA	n.p.	n.g.	positive	Positive in both cell types.	NTP (1995)

Abbreviations: HID = highest ineffective dose; LED = lowest effective dose; n.p. = not provided; n.g. = not given; NA = not applicable

Figure 5-1. Genetic Activity Profile of Danthron (Data limited to IARC, 1990)

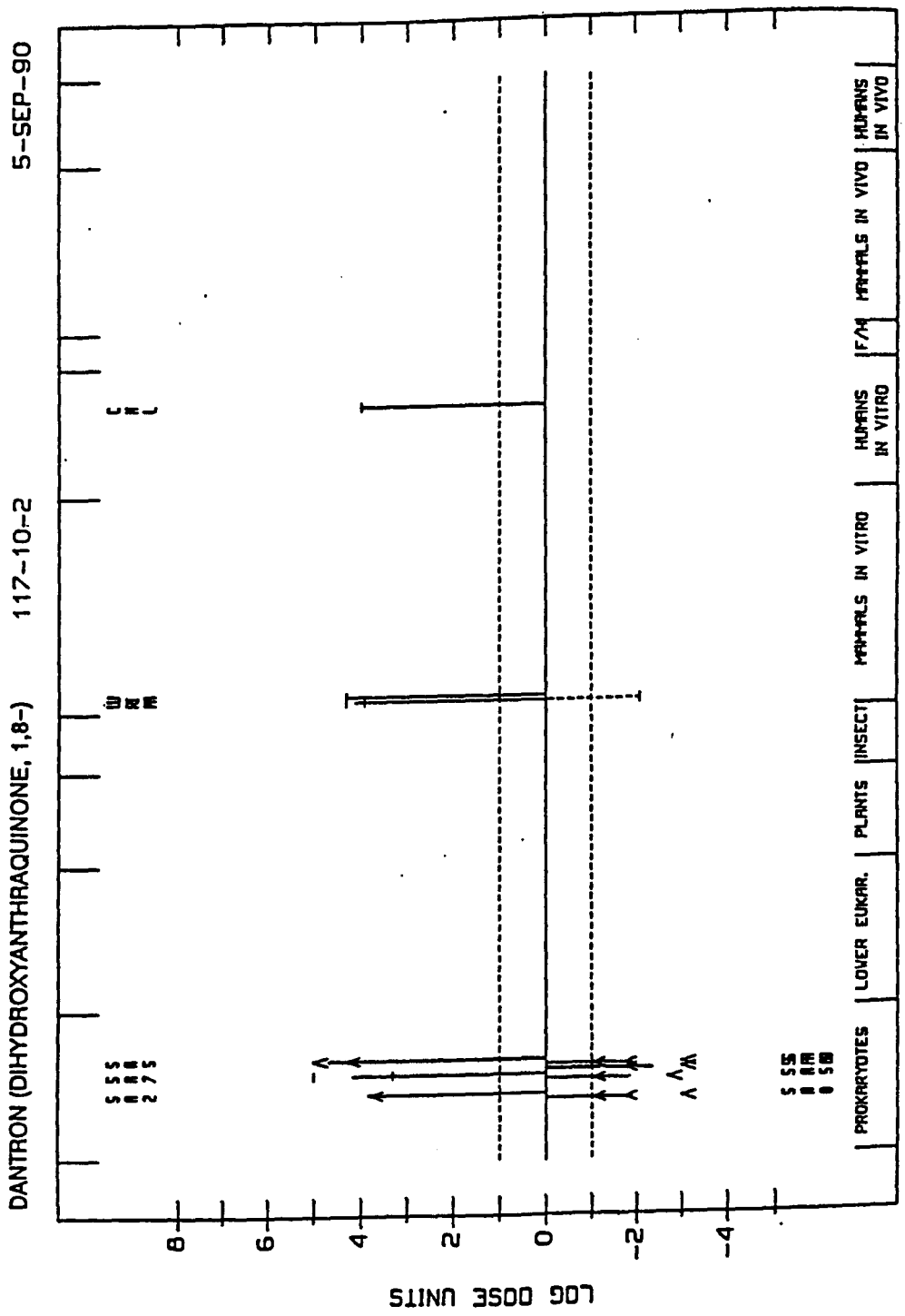
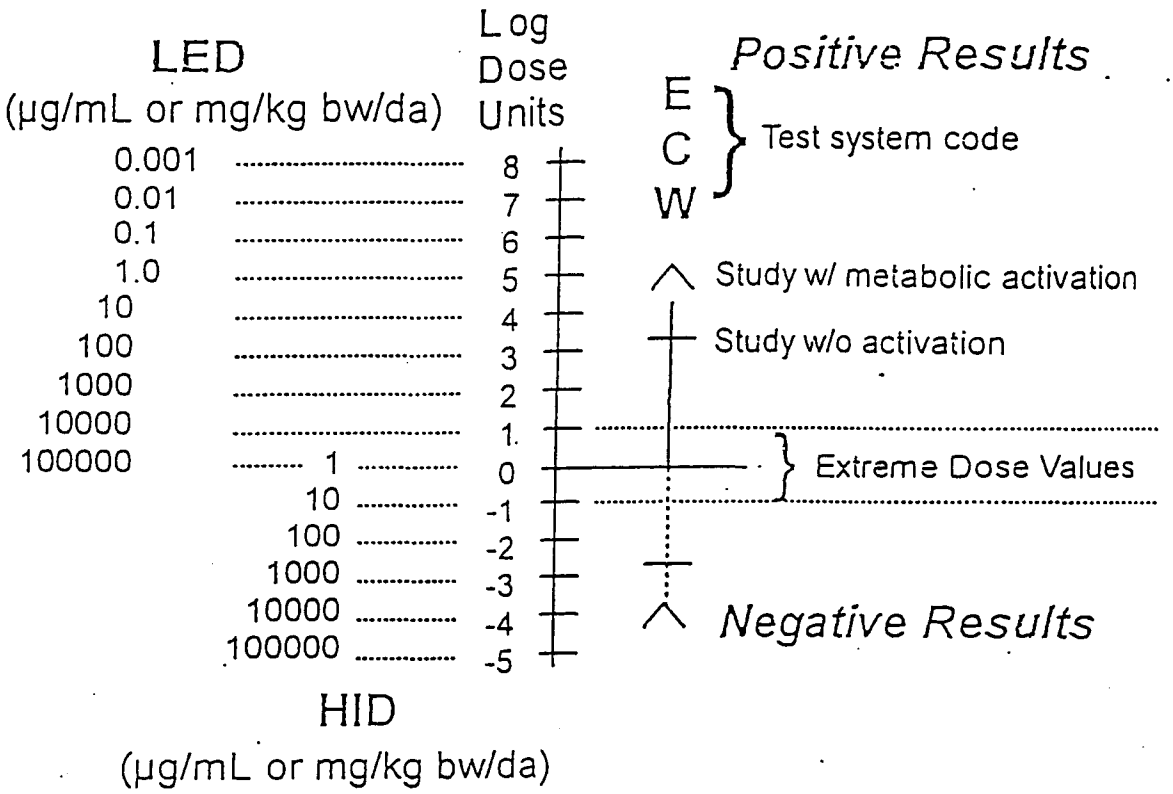


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N.E., H.F. Stack, M.R. Gross, and M.D. Waters. 1984. An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. Mutat. Res. 143:89-111.

Waters, M.D., H.F. Stack, A.L. Brady, P.H.M. Lohman, L. Haroun, and H. Vainio. 1988. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutat. Res. 205:295-312.

Waters, M.D., H.F. Stack, N.E. Garrett, and M.A. Jackson. 1991. The genetic activity profile database. Environ. Health Perspect. 96:41-45.

6.0 OTHER RELEVANT DATA

6.1 Absorption, Distribution, Metabolism, and Excretion

Summary: Danthron administered directly into the duodenum via a non-recirculating perfusion system was significantly absorbed from rat duodenum. In rats, danthron was absorbed in the upper gastrointestinal tract, thereby preventing substantial amounts of drug from reaching the site of therapeutic action in the colon. Following absorption in the duodenum, glucuronide and sulfate conjugates were formed. Danthron or conjugated products were not found in several organs examined 48 h after administration of the dose to rats. It is thought that bacterial reduction of danthron in the colon did not play a major role in the metabolism of this compound. Several metabolites of danthron were detected in the bile and urine of rats i.v. administered this compound, including the monosulfate, β-glucuronide, two diconjugates (tentative), and several phase I metabolites. Only 30 to 50% of the dose was accounted for by conjugates in the bile and urine. P-450 is directly involved in the reduction of danthron. When compared to other anthraquinones such as doxorubicin ($V/V_o = 1.11$) and emodin ($V/V_o = 1.22$), danthron was the most actively reduced ($V/V_o = 1.7$) as suggested by the ratio of maximal semiquinone formation rate to oxygen-catalyzed rate (V/V_o). The monoglucuronide and monosulfate of danthron were formed *in vitro* following 1-h exposure of everted sacs of rat jejenum and colon on the mucosal (luminal) side to danthron. Danthron was absorbed transplacentally and excreted via the fetal kidney into the amniotic fluid following administration to pregnant women the evening before the induction of labor; and there is strong evidence of milk transfer of danthron in humans.

Male Wistar rats under urethan anesthesia had part of their duodenum cannulated and the bile duct ligated. Danthron (1.2 mg/L) administered directly into the duodenum via a non-recirculating perfusion system (150 mM choline chloride, buffered at pH 7.4 with Tris HCl) was absorbed from rat duodenum to a significant extent. The results indicated that anthraquinone aglycones are absorbed in the upper gastrointestinal tract, thereby preventing substantial amounts of drug from reaching the site of therapeutic action in the colon (Breimer and Baars, 1976).

In subsequent studies using female rats administered danthron (2.0 to 6.1 mg) orally either as an aqueous suspension or homogenized in a biscuit, only 40% of the administered dose was collected as parent compound and sulfate and glucuronide conjugates in urine and feces within 48 h (Breimer and Baars, 1976).

Orange danthron is excreted in the urine as a red dye, which may discolor the skin (Martin and Cook, 1961; IARC, 1990).

In agreement with the cannulated duodenum experiments mentioned above, urine results showed that glucuronide and sulfate conjugates were formed following absorption in the duodenum. Small amounts of other conjugates were formed, which were detected following alkaline or acid hydrolysis after enzymatic hydrolysis with β-glucuronidase or sulfatase. Sequestration of danthron or conjugated products was not found in several organs examined 48 h after administration of the dose. The likelihood that bacterial reduction in the colon played a role in the metabolism of danthron and accounted for any of the unrecovered administered dose was low, since anthrone (1,8-dihydroxy-9-anthrone [anthralin]) or 1,8-dihydroxy-10-anthrone would have been oxidized to parent compound during extraction and chemical hydrolysis (Breimer and Baars, 1976).

After male Wistar rats were given danthron by i.v. infusion as the sodium salt in doses of 4.8, 22, or 58 μmol/kg bw, several metabolites were detected in the bile and urine, including the monosulfate, β-glucuronide, two diconjugates (tentative), and several phase I metabolites. Approximately 80% of the danthron conjugates in bile were excreted after 1 h following i.v.

infusion. The dose fractions in the bile of the low-, mid-, and high-dose levels represented approximately 20%, 30%, and 40%, respectively, after 5 h. In urine, the corresponding fractions of the doses were 16%, 12%, and 10%, with bile:urine excretion ratios of 1.3, 2.7, and 4.0, respectively. Only 30 to 50% of the dose was accounted for by conjugates in the bile and urine (Sund, 1987).

When the rats were administered 120 μmol/kg bw by gavage, about 0.5% of the dose was excreted as conjugates per hour for at least 6 h. Initially, excretion in bile was higher than in urine; but in later periods, urinary excretion exceeded bilirary excretion. Within 6 h, cumulative excretion in bile plus urine was about 6% of the original dose (Sund, 1987).

The monoglucuronide and monosulfate of danthron were formed *in vitro* following 1-h exposure of everted sacs of rat jejenum and colon on the mucosal (luminal) side to danthron. The monoglucuronide was the major *in vitro* metabolite. Both conjugates concentrated on the luminal side in the jejenum. In the colon, the monoglucuronide accumulated more on the blood side (Sund and Elvegard, 1988).

6.1.1 Transplacental Passage

Blair et al. (1977) administered danthron (in the form of Dorbanex containing 25 mg danthron) to pregnant women (12) the evening before the induction of labor. Maternal urine samples and amniotic fluid were collected approximately 12 h after administration of the drug and the neonatal urine samples were collected up to 20 h after administration of the dose. Danthron was found in the babies' urine immediately after delivery, with a much lower concentration detectable in the amniotic fluid. The findings suggested that danthron was absorbed transplacentally and excreted via the fetal kidney into the amniotic fluid (Blair et al., 1977). The average proportion of danthron found in maternal and neonatal urine present as conjugate was 95% and 94%, respectively. The average proportion of total conjugated danthron in the mothers was 97%, with a slightly lower proportion found in the babies (87%). These data suggested that glucuronidation is the preferred method of elimination in mother and baby, although in the baby a somewhat wider scatter of results was found.

6.1.2 Transfer of Danthron in Human Milk

Anthraquinone cathartics ingested by lactating women were well known to be transferred to their breast-fed infants in amounts sufficient to exert a cathartic action in the infants (Martin and Cook, 1961).

Giroux et al. (1992) listed danthron specifically as a chemical with strong evidence of milk transfer in humans. Data related to, in order of decreasing importance, excretion in humans, excretion in animals, detection in humans, and detection in animals were used to evaluate the evidence of milk transfer in humans. Giroux et al. (1992) extracted data from the Quebec *Commission de la Santé et de la Securité du Travail* [Occupational Health and Safety] (CSST; Infotox database) computer system that provides peer-reviewed information on 5500 chemicals identified in the workplace that may appear in the mother's milk.

6.1.3 Phase-I Enzymes Involved in Danthron Metabolism

P-450 is directly involved in the reduction of danthron (Chesis et al., 1984). When aerobically incubated with purified S9, danthron was found to be activated to a highly mutagenic species in *S. typhimurium* TA104 by the cytochrome P-450 monooxygenase system as determined by the inhibition of mutagenicity following the addition of SKF525A (a potent

cytochrome P-450 inhibitor) and the finding that this quinone was found to be a very poor redox cycler (P-450 reductase); that is, danthron did not undergo extensive oxidation-reduction reactions wherein oxygen free radicals are generated. When danthron was incubated with purified P-450 reductase and S9, it was slightly mutagenic to *S. typhimurium* when compared to incubations including S9 and danthron. In addition, when dicoumarol (selectively inhibits DT-diaphorase) was added to S9 preparations containing danthron, inhibition of mutagenicity was not evident. The findings suggest that the *in vitro* mutagenicity of danthron (see above) is due to the metabolism of danthron to a highly mutagenic metabolite catalyzed primarily by cytochrome P-450 monooxygenase (Chesis et al., 1984).

The relative rate of superoxide ($O_2 \cdot^-$) formation was quantitated by measuring the SOD-inhibitable reduction of succinylated cytochrome C, which was found to be in direct agreement with the relative mutagenicity (poor/slight vs. S9 incubations discussed above) of danthron incubated with purified NADPH P-450 reductase. The authors suggested that the slight mutagenicity observed in incubations including purified P-450 reductase was due to the one-electron reduction of danthron to semiquinones, formation of superoxide, and, subsequently, formation of hydrogen peroxide (H_2O_2) (Chesis et al., 1984).

Danthron was poorly metabolized in an *in vitro* xanthine oxidase/hypoxanthine/catalase system as measured by cytochrome C reduction and semiquinone metabolite production. The rate of cytochrome C reduction stimulated by danthron in the *in vitro* system ranged from 0.74 to 0.94 nmol/min when incubated with 10 mM to 75 mM, respectively, compared to 0.62 nmol/min in the controls. When compared to other anthraquinones such as doxorubicin ($V/V_o = 1.11$) and emodin ($V/V_o = 1.22$), danthron was the most actively reduced ($V/V_o = 1.7$) as suggested by the ratio of maximal semiquinone formation rate to oxygen-catalyzed rate (V/V_o). In agreement with V/V_o rates, danthron displayed the greatest maximal anthraquinone-stimulated cytochrome C reduction in the presence of excess compound (K_m): danthron (6.7 mM) > emodin (2.4 mM) > doxorubicin (1.2 mM) (Lewis and Shibamoto, 1989).

6.2 Pharmacokinetics

No data were found.

6.3 Modes of Action

Summary: Danthron was found to be genotoxic in a limited number of prokaryotic, lower eukaryotic, and mammalian *in vitro* test systems. When incubated with purified S9, danthron was found to be activated to a highly mutagenic species by the cytochrome P-450 monooxygenase system in *S. typhimurium* TA104. Superoxide was formed by illuminated danthron in the presence of dissolved oxygen and the reducing agent ergothioneine. "It has been known for some time that anthraquinones in aerobic aqueous solutions are susceptible to photomediated reactions, ultimately generating active species that can lead to hydroxylation of the anthraquinones" (Broadbent, 1967; Bruce, 1974; both cited by Hartman and Goldstein, 1989).

6.3.1 Genotoxicity

Danthron was found to be genotoxic in a limited number of prokaryotic, lower eukaryotic, and mammalian *in vitro* test systems. (See Section 5.0.) Danthron induced gene mutations in several strains of *S. typhimurium* in the presence and/or in the absence of S9 metabolic activation, induced respiration-deficient mutations in the yeast *S. cerevisiae*, and

inhibited gap-junction intercellular communication in Chinese hamster V79 cells; however, this latter effect was not reproducible in another V79 cell study as well as in human fibroblasts. UDS in both mouse and rat primary hepatocytes and chromosomal aberrations in human peripheral blood lymphocytes in the absence of exogenous metabolic activation were also induced by danthron exposure.

6.3.2 Metabolism and Genotoxicity

When incubated with purified S9, danthron was found to be activated to a highly mutagenic species by the cytochrome P-450 monooxygenase system in *S. typhimurium* TA104. (See Section 6.1.3.) However, these data were generated using purified NADPH-cytochrome P-450 reductase, which does not provide for the role of glutathione-*S*-transferase in the detoxification of danthron. The role of glutathione in protecting against quinone mutagenicity therefore requires further study (Chesis et al., 1984).

Subsequently, Hartman and Goldstein (1989) studied superoxide generation by several illuminated (broad spectrum wavelength) anthraquinones, including danthron, as measured by the reduction of nitroblue tetrazolium in the absence and presence of superoxide dismutase. Superoxide was formed by illuminated danthron in the presence of dissolved oxygen and the reducing agent ergothioneine. Minimal or no reduction of nitroblue tetrazolium was observed in the absence of illumination or in the absence of ergothioneine. "It has been known for some time that anthraquinones in aerobic aqueous solutions are susceptible to photomediated reactions, ultimately generating active species that can lead to hydroxylation of the anthraquinones" (Broadbent, 1967; Bruce, 1974; both cited by Hartman and Goldstein, 1989).

The facile generation of superoxide demonstrated by Hartman and Goldstein (1989) mimics conditions common to a number of *in vitro* biological systems. "Therefore, external production of superoxide can readily be pictured as a mutagen by such secondary means" (Hartman and Goldstein, 1989).

6.4 Structure-Activity Relationships

Summary: Emodin, a structural analogue of danthron, was incapable of binding to DNA at neutral pH when incubated with calf thymus DNA, but was found to be mutagenic to several strains of *S. typhimurium* only after metabolic activation. The genotoxicity of emodin was negative in mammalian systems for the induction of SCE and HPRT forward mutations in Chinese hamster V79 cells and was also negative for mutagenicity in the hepatocyte HPC/DNA repair test. Another structural analogue of danthron, aloe-emodin glucoside, induced chromosomal aberrations in mitosing cells of *Vicia fabia*, including subchromatid breaks and rearrangements. Computer automated structure evaluation (CASE) identified one active fragment (HO-C=) that had a high probability ($P < 0.05$) of being associated with carcinogenesis in rodents.

Only phenolic anthraquinone purgatives (present in aloe, cascara sagrada, rhubarb, and senna) were considered for this discussion. These compounds include emodin, aloe-emodin, isoemodin, and chrysophanic acid and have hydroxyl, methyl, and/or hydroxymethyl group substituents on the anthraquinone rings. Note that several other anthraquinones that have reactive groups (i.e., amino and nitro) were found to be carcinogenic to rodents: 2-aminoanthraquinone (NCI, 1978a), 1-amino-2-methylanthraquinone (NCI, 1978b), 2-methyl-1-nitroanthraquinone (NCI, 1978c), and Disperse Blue 1 (NTP, 1986).

The mutagenicity of phenolic anthraquinones has been reviewed by Sendelbach (1989) and Brown (1980; cited by Sendelbach, 1989) and discussed below. Sendelbach (1989) provided a brief literature summary on the mutagenicity of anthraquinone derivatives since the review of Brown (1980). An extensive on-line search for references pertaining to the carcinogenicity and mutagenicity of danthron structural analogues has not been conducted.

6.4.1 Mutagenicity of Structural Analogues (Emodin and Aloe-Emodin)

Emodin (1,3,8-trihydroxy-6-methyl-9,10-anthracenedione) was incapable of binding to DNA at neutral pH when incubated with calf thymus DNA (Swanbeck, 1966; cited by Sendelbach, 1989). In *Salmonella* strains TA90, TA97, TA102, TA1537, TA2637, emodin was mutagenic after activation (Swanbeck, 1966; Brown and Brown, 1976; Wehner et al., 1979; Brown, 1980; all cited by Sendelbach, 1989). Emodin and other phenolic anthraquinones did not damage DNA, as assessed by repair tests in the *Bacillus subtilis* rec M45 assay (Kada et al., 1983; cited by Sendelbach, 1989). Emodin was negative in mammalian systems for the induction of SCE and HPRT forward mutations in Chinese hamster V79 cells (Bruggeman and van der Hoeven, 1984; cited by Sendelbach, 1989). Emodin was also negative for mutagenicity in the hepatocyte HPC/DNA repair test (Kawai et al., 1984; cited by Sendelbach, 1989).

Aloe-emodin (1,8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione) glucoside induced chromosomal aberrations in mitosing cells of *V. faba*, including subchromatid breaks and rearrangements (Schmid, 1956; cited by Sendelbach, 1989).

6.4.2 Structural Alert Identification

Fu et al. (1995) analyzed 233 rodent carcinogens, including danthron, from the Carcinogenic Potency Database (CPDB; Gold et al., 1991; cited by Fu et al., 1995) with CASE (Computer Automated Structure Evaluation), and compared the extents of target organs (single or multiple site) with the sensitivities for long-term carcinogenic bioassays in rodents, Salmonella assay ("Sty"; Gold et al., 1993; cited by Fu et al., 1995), electrophilic substructure alert analysis (ESAA; Ashby and Paton, 1993; cited by Fu et al., 1995) and CASE. Fu et al. (1995) listed danthron as an organ-specific carcinogen (site not mentioned) and CASE identified one active fragment (HO-C=) that had a high probability ($P < 0.05$) of being associated with carcinogenesis in rodents. In brief, mutagenic or electrophilic carcinogens were more likely to induce tumors at multiple organs; in contrast, most carcinogens that induced tumors in a single target organ in one species were rarely mutagenic or electrophilic. The results showed that danthron was negative for ESAA. Fu et al. (1995) did not list the mutagenicity (Sty) of danthron; however, it has been found to be genotoxic in several strains of *S. typhimurium* in the presence and/or in the absence of S9 metabolic activation (See Section 5.0).

6.5 Cell Proliferation

Experimental details for the studies described in this section are presented in Table 6-1.

Summary: The incidences of adenomatous hyperplasia of the cecum and colon were significantly increased in male mice (females not evaluated) administered 2000 ppm danthron in the diet for up to 540 days. In male rats (females not evaluated), significant positive correlations were detected between BrdU-labeling indices in the small intestine, cecum, and colorectum (proximal, middle, and distal portions) and danthron dose (125-4000 ppm in the diet for 7 days).

6.5.1 Mice

The incidences of adenomatous hyperplasia of the cecum and colon were significantly increased in male C3H/HeN mice (females not evaluated) administered 2000 ppm danthron in the diet beginning at 8 weeks of age for up to 540 days (Mori et al., 1986).

6.5.2 Rats

Significant positive correlations were detected between BrdU-labeling indices in the small intestine, cecum, and colorectum (proximal, middle, and distal portions) and danthron dose in male F344/DuCrj rats (females not evaluated) administered 125, 250, 500, 1000, 2000, or 4000 ppm danthron in the diet beginning at 6 weeks of age for 7 days. Labeling indices were only measured at 7 days; there were no intermediate measurements (Toyoda et al., 1994).

"Focal hyperplastic lesions of the glandular epithelium of the colon and cecum were frequently encountered" in male ACI rats (females not evaluated) administered 10,000 ppm danthron in the diet beginning at 8 weeks of age for 16 months. No other details were given (e.g., incidence or severity) (Mori et al., 1985).

6.6 Initiation/Promotion

Experimental details for the studies described in this section are presented in Tables 6-2 and 6-3.

Summary: In a study that evaluated the effects of co-administered 7,12-dimethylbenz[*a*]anthracene (DMBA) and danthron, no skin tumors were detected in female mice (males not evaluated) that had danthron or danthron in combination with DMBA applied on skin for up to 476 days. In studies that evaluated the synergistic effects of 1,2-dimethylhydrazine (DMH) and danthron, the incidence and multiplicity of adenoma of the colon and adenoma of the liver were significantly increased in male mice (females not evaluated) administered DMH subcutaneous (s.c.) once/wk for 12 weeks followed by 2000 ppm danthron in the diet for 42 weeks. The incidence of adenocarcinoma of the colon and carcinoma of the liver, however, were not significantly increased. Mice that received danthron alone or saline alone did not develop colon tumors. In male rats (females not evaluated) that were administered both DMH and danthron (a single s.c. dose of DMH, followed 1 week later with 600 or 2400 ppm danthron in the diet for 25 weeks), there was no significant increase in the combined incidence of intestinal adenoma and adenocarcinoma. Rats that received danthron alone or were left untreated did not develop intestinal adenocarcinoma; their incidence of adenoma was not given.

There was no significant increase in the multiplicity or area of glutathione *S*-transferase placental form (GST-P)-positive foci in the liver of male rats (females not evaluated) administered diethylnitrosamine (DEN; single s.c. injection of 200 mg/kg bw) and danthron (2000 ppm in diet beginning 2 weeks after DEN injection and continuing for 6 weeks). In another study, adenomatous hyperplasia of the cecum was detected in all male mice (females not evaluated) administered DMH and danthron or saline and danthron, but in none of the DMH or saline controls. DMH (20 mg/kg bw) or saline was administered s.c. for 12 weeks. Danthron administration (2000 ppm in the diet) was begun 1 week after the last injection of DMH or saline and was continued for an additional 42 weeks.

6.6.1 Mammalian Carcinogenicity

6.6.1.1 7,12-Dimethylbenz[*a*]anthracene (DMBA) and Danthron

In a two-stage carcinogenesis study, no skin tumors were detected in female ICR/Ha Swiss mice (males not evaluated) administered DMBA + danthron (single skin application of 20 μg DMBA in 0.1 mL acetone, followed 2 weeks later with 170 μg [0.71 μmol] danthron in 0.1 mL acetone on skin 3 times per week) or danthron alone, beginning at 7 weeks of age, for up to 476 days (Segal et al., 1971; cited by IARC, 1990).

6.6.1.2 1,2-Dimethylhydrazine (DMH) and Danthron

Sugie et al. (1994) evaluated the promoting effect of danthron. The incidence and multiplicity of adenoma of the colon and adenoma of the liver were significantly increased in male ICR/CD-1 mice (females not evaluated) administered DMH + danthron (20 mg DMH/kg bw s.c. once/wk for 12 weeks, followed 1 week later with 2000 ppm danthron in the diet for 42 additional weeks), beginning at 6 weeks of age, as compared to DMH controls. The incidence of adenocarcinoma of the colon and carcinoma of the liver were not significantly increased in mice that received DMH + danthron. Mice that received danthron alone or saline alone did not develop colon tumors. Adenoma of the liver was detected in 2/28 mice that received danthron alone (vs. 1/30 saline controls), but the statistical significance of this was not specified.

In a two-stage carcinogenesis study, there was no significant increase in the combined incidence of intestinal adenoma and adenocarcinoma in male Sprague-Dawley rats (females not evaluated) that were administered DMH + danthron (150 mg DMH/kg bw administered as a single s.c. dose, followed 1 week later with 600 or 2400 ppm danthron in the diet for 25 weeks). Rats that received danthron alone or were left untreated did not develop intestinal adenocarcinoma. The incidence of adenoma in these rats was not given (Sjöberg et al., 1988; cited by IARC, 1990).

6.6.2 Cell Proliferation

6.6.2.1 Diethylnitrosamine (DEN) and Danthron

There was no significant increase in the multiplicity (number/cm^2) or area (mm^2/cm^2) of glutathione *S*-transferase placental form (GST-P)-positive foci in the liver of male F344 rats (females not evaluated) administered DEN and danthron beginning at 6 weeks of age. DEN (200 mg/kg bw) was administered as a single intraperitoneal injection. Danthron administration (2000 ppm in diet) was begun 2 weeks after the DEN injection and was continued for an additional 6 weeks. At week 3 of danthron administration, all rats were subjected to a two-thirds partial hepatectomy. All rats were killed at 8 weeks (Hasegawa and Ito, 1992).

6.6.2.2 1,2-Dimethylhydrazine (DMH) and Danthron

In a study conducted by Sugie et al. (1994), adenomatous hyperplasia of the cecum was detected in all male ICR/CD-1 mice (females not evaluated) administered DMH and danthron or saline and danthron, but in none of the DMH or saline controls. DMH (20 mg/kg bw) or saline (20 mg/kg bw) was administered s.c. once/wk for 12 weeks, beginning at 6 weeks of age. Danthron administration (2000 ppm in the diet) was begun 1 week after the last injection of DMH or saline and was continued for an additional 42 weeks.

Table 6-1. Cell Proliferation Induced by Danthron

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice – *Oral Administration*							
8-wk-old C3H/HeN mouse	20M	20M (basal diet alone)	danthron, no impurities detected on thin-layer chromatography	2000 ppm in diet	up to 540 days	Mice were killed when moribund or at the end of the treatment period. The mean survival time and mean body weight of danthron-treated mice did not differ significantly from those of controls. **Cecum:** Positive (for proliferative activity, as indicated by presence of hyperplasia) The incidence of adenomatous hyperplasia was significantly increased in danthron-treated mice (17/17 vs. 0/19 controls [$p < 0.001$, Fisher's exact test]). **Colon:** Positive (for proliferative activity, as indicated by presence of hyperplasia) The incidence of adenomatous hyperplasia was significantly increased in danthron-treated mice (5/17 vs. 0/19 controls [$p < 0.02$, Fisher's exact test]).	Mori et al. (1986)
Rats - *Oral Administration*							
6-wk-old F344/DuCrj rat	4M per dose	groups of 4M (basal diet alone)	danthron, purity not specified	125, 250, 500, 1000, 2000, or 4000 ppm in diet	7 days	All rats received a single i.p. injection of BrdU (40 mg/kg bw) 2 hours before being killed on day 7 of the experiment. At necropsy, the intestines were removed and evaluated for BrdU incorporation. Numbers of BrdU-labeled cells were counted microscopically in 5 regions (proximal portion of the small intestine, the cecum, and proximal, middle, and distal portions of the colorectum). BrdU labeling indices were expressed as the number of labeled cells per 5 crypts in each region of the rat intestine. Statistical significance was determined using Student's or Cochran's *t*-test and correlation analysis. **Small Intestine, Cecum, Colorectum:** Positive (for proliferative activity, as indicated by BrdU-labeling index) Significant positive correlations were detected between BrdU-labeling indices and dose in the small intestine ($p < 0.01$), cecum ($p < 0.001$), and colorectum (proximal: $p < 0.001$; middle: $p < 0.001$; distal: $p < 0.001$) of danthron-treated rats.	Toyoda et al. (1994)
8-wk-old ACI rat	18M	15M (basal diet alone)	danthron, "pure"	10,000 ppm in diet	16 mo	Rats were killed when moribund or at the end of the treatment period. **Intestinal Tract:** "Focal hyperplastic lesions of the glandular epithelium of the colon and cecum were frequently encountered" in both danthron-treated rats with intestinal tumors and those without intestinal tumors, but in none of the controls. No other details about intestinal hyperplasia were given (e.g., incidence or severity).	Mori et al. (1985)

Abbreviations: i.p. = intraperitoneal; M = male(s)

Table 6-2. Mammalian Carcinogenicity of Danthron in Combination with Other Treatments

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
7, 12-Dimethylbenz[*a*]anthracene (DMBA) and Danthron							
7-wk-old ICR/Ha Swiss mouse	20F (DMBA + danthron	20F (danthron alone)	danthron, purity not specified (commercial grade)	170 µg (0.71 µmol) in 0.1 mL acetone, 3 skin applications/ wk	490 days	DMBA (20 µg in 0.1 mL acetone) was administered as a single skin application (Initiation). Danthron administration was begun 2 weeks after the DMBA application and was continued for an additional 476 days (Promotion). **Skin:** Negative No skin tumors were detected in either group.	Segal et al. (1971; cited by IARC, 1990)
1,2-Dimethylhydrazine (DMH) and Danthron							
6-wk-old ICR/CD-1 mouse	30M (DMH + dathron) (Group 2) 30M (saline + danthron) (Group 3)	30M (DMH alone) (Group 1) 30M (saline alone) (Group 4)	danthron, purity not specified	2000 ppm in diet	54 wk	Mice in Groups 1 and 2 were administered DMH (20 mg/kg bw) s.c. once/wk for 12 weeks. In Groups 3 and 4, saline (20 mg/kg bw) was administered for 12 weeks instead of DMH. In Groups 2 and 3, danthron administration was begun 1 week after the last injection of DMH (Group 2) or saline (Group 3) and was continued for an additional 42 weeks. "All organs were examined grossly and the location and number of tumors in any organs, especially intestines and liver, were recorded." Sections of intestines and liver with and without tumors were examined histologically. **Colon:** Negative (with danthron alone) Positive (for adenoma with **previous DMH treatment**) None of the rats that received danthron alone or saline alone developed colon tumors. The incidence and multiplicity of adenoma were significantly increased in Group 2 as compared to Group 1 (13/23 vs. 6/29 DMH controls [$p < 0.01$, χ^2]; 1.17 $\pm$ 1.63 tumors/mouse vs. 0.24 $\pm$ 0.50 tumors/mouse in DMH controls [$p < 0.02$, Student's t-test]). The incidence of adenocarcinoma was not significantly increased in mice that received DMH + danthron. **Liver:** None of the mice that received danthron alone developed carcinoma. Adenoma was detected in 2/28 mice that received danthron alone (vs. 1/30 saline controls), but the statistical significance of this was not specified. The incidence and multiplicity of adenoma were significantly increased in Group 2 as compared to Group 1 (13/23 vs. 4/29 DMH controls [$p < 0.002$, Fisher's exact test]; 1.22 $\pm$ 1.91 tumors/mouse vs. 0.21 $\pm$ 0.55 tumors/mouse in DMH controls [$p < 0.02$, Student's t-test]). The incidence of carcinoma was not significantly increased in mice that received DMH + danthron, as compared to DMH controls.	Sugie et al. (1994)

Table 6-2. Mammalian Carcinogenicity of Danthron in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
50-day-old Sprague-Dawley rat	30M (DMH + LD danthron) 30M (DMH + HD danthron) 30M (danthron alone)	30M (basal diet alone) 30M (DMH alone)	danthron, >97% pure	600 or 2000 ppm in diet (~30 or 60 mg/kg bw [125 or 250 µmol/kg bw] average daily intake)	25 wk	DMH alone (150 mg/kg bw) was administered as a single s.c. injection. Danthron administration was begun 1 week after DMH administration. There was no significant difference in mean body weight gain between treatment groups. It was not specified in the IARC review which statistical tests were used. **Intestinal Tract:** Negative None of the rats that received danthron alone or were untreated developed intestinal adenocarcinoma. The incidence of adenoma in these rats was not given. There was no significant increase in the combined incidence of intestinal adenoma and adenocarcinoma in rats that received DMH + danthron (4/30 LD and 2/30 HD rats vs. 2/30 DMH controls). Intestinal tumor incidence was not broken down by tumor type for these groups.	Sjöberg et al. (1988; cited by IARC, 1990)

Abbreviations: F = female(s); HD = high dose; LD = low dose; M = male(s); MD = mid dose; s.c. = subcutaneous(ly)

Table 6-3. Cell Proliferation Induced by Danthron in Combination with Other Treatments

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Diethylnitrosamine (DEN) and Danthron							
6-wk-old F344 rat	15M (DEN + danthron) M, no. not provided (NaCl + danthron)	13M (DEN + basal diet)	danthron, purity not specified	2000 ppm in diet	6 wk	DEN (200 mg/kg bw in 9% [w/v] NaCl) or NaCl alone was administered as a single i.p. injection. Danthron administration was begun 2 weeks after the DEN or NaCl injection and was continued for an additional 6 weeks. At week 3 of danthron or basal diet administration, all rats were subjected to a two-thirds partial hepatectomy. All rats were killed at 8 weeks. Three slices of the liver, one each from the right posterior and caudate lobes, and one from the right anterior lobe, were fixed in ice-cold acetone for immunohistochemical examination of glutathione *S*-transferase placental form (GST-P) positive foci. Statistical analyses were performed using "Student's t-test and Welch's t-test in combination with the F-test for variability." **Liver:** Negative There was no significant increase in the multiplicity (number/cm^2) or area (mm^2/cm^2) of GST- P-positive foci in the liver of rats treated with DEN + danthron as compared to rats treated with DEN alone. No data were presented for rats that were treated with NaCl + danthron.	Hasegawa and Ito (1992)
1,2-Dimethylhydrazine (DMH) and Danthron							
6-wk-old ICR/CD-1 mouse	30M (DMH + danthron) (Group 2) 30M (saline + danthron) (Group 3)	30M (DMH alone) (Group 1) 30M (saline alone) (Group 4)	danthron, purity not specified	2000 ppm in diet	54 wk	Mice in Groups 1 and 2 were administered DMH (20 mg/kg bw) s.c. once/wk for 12 weeks. In Groups 3 and 4, saline (20 mg/kg bw) was administered for 12 weeks instead of DMH. In Groups 2 and 3, danthron administration was begun 1 week after the last injection of DMH (Group 2) or saline (Group 3) and was continued for an additional 42 weeks. **Cecum:** Positive (for proliferative activity, as indicated by presence of hyperplasia) Adenomatous hyperplasia was detected in all DMH + danthron-treated and all danthron-treated mice (23/23 and 28/28, respectively, vs. 0/29 DMH controls and 0/30 saline controls; no p-values given).	Sugie et al. (1994)

Abbreviations: i.p. = intraperitoneal; M = male(s); s.c. = subcutaneous(ly)

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APPENDIX A

DESCRIPTION OF ONLINE LITERATURE SEARCHES FOR DANTHRON

DESCRIPTION OF ONLINE SEARCHES FOR DANTHRON (IARC Monograph in Vol. 50, 1990)

The searches described below were conducted between March and October 1996. An exhaustive search of all pertinent databases was not attempted, but the ones chosen were expected to provide citations for most of the relevant recently published literature. No attempt was made in the search strategy to find toxicity information for metabolites and other structural analogues.

Generally, if an IARC monograph or another authoritative review had been published, literature searches were generally restricted from the year before publication to the current year.

Older literature that needed to be examined was identified from the reviews and original articles as they were acquired. Current awareness was maintained by conducting weekly searches of Current Contents on Diskette® Life Sciences 1200 [journals] edition.

TOXLINE (on STN International): Use of the synonyms danthron, dantron, and dihydroxyanthraquinone (unqualified) retrieved a total of 184 records. After examination of the record titles and some full citations, 44 publications were selected for acquisition.

CTCP (CLINICAL TOXICOLOGY OF COMMERCIAL PRODUCTS): Online database available from the Chemical Information System, last updated in 1985.

EMIC/EMICBACK: Forty-nine records were indexed by the CASRN.

IRIS: No profile was found in this EPA risk assessment database.

TSCAPP (TSCA PLANT AND PRODUCERS): Eight records were indexed by CASRN representing importers or manufacturing plants. EPA database available from the Chemical Information System, last updated in 1983; primarily represents the 1977 Toxic Substances Control Act Inventory.

In September 1996, the contractor performed searches for updating sections 1 and 2, which had been last updated in 1994 with regulatory information from print sources and REGMAT (May 1993 version). REGMAT had broad coverage of EPA regulations, but it is no longer available. Databases searched in 1996 included CSCHEM and CSCORP for U.S. suppliers (databases produced by Chem Sources); HSDB; the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System) and ISHOW (for physical-chemical properties); Chemical Abstracts Service's (CAS) File CHEMLIST for TSCA and SARA updates in 1996; PHIND (the Pharmaceutical and Healthcare Industry News Database, DIALOG File 129); and CAS's CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information.

In further attempts to identify pertinent FDA regulations and the current usage status (approved or investigational), another series of searches in September 1996 was performed in pharmaceuticals and other regulatory databases. The databases included the following:

- 21 CFR (via Internet access)
- Clinical Pharmacology (drug monographs available on the Internet from Gold Standard Multimedia Inc.)
- Derwent Drug File (DIALOG File 376 for nonsubscribers) (covers 1964-1982)
- Diogenes (DIALOG File 158) (covers 1976-1996; file includes FDA regulatory information from news stories and unpublished documents, including listings of approved products, documentation of approval process for specific products, recall, and regulatory action documentation)
- Drug Information Fulltext (DIALOG File 229) (current, updated quarterly; includes information on at least 1000 commercially available drugs and 57 investigational injectable drugs)
- Federal Register (DIALOG File 669) (covers 1988-1996) (full text)
- Federal Register Abstracts (DIALOG File 136) (covers 1977-1993)
- International Pharmaceutical Abstracts (DIALOG File 74) (covers 1970-1996, all phases of drug development including laws and state regulations)
- NCI/PDQ. National Cancer Institute's menu-driven online database available from the National Library of Medicine and via the Internet. File contains state-of-the-art cancer treatment protocols and clinical trials. 1996

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Chromosomal aberrations, human lymphocytes in vivo

Test Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test Code	Definition
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test Code	Definition
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange