DRAFT PRIORITIZED CANDIDATE CHEMICALS UNDER CONSIDERATION FOR CARCINOGENICITY EVALUATION:

BATCH #4

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

October 2003

Draft data summaries for 50 chemicals under consideration for carcinogenicity evaluation ("Batch #4") have been prepared and are presented here. The same process utilized to select the previous group of chemicals prioritized for carcinogenicity concern (*i.e.*, Batch #3) was employed in the selection of the Batch #4 chemicals. Namely, 50 chemicals were randomly selected from 100 chemicals in the tracking database. The 100 chemicals consisted of the 39 chemicals remaining from the previous random selection and 61 additional chemicals selected using a table of random numbers from among those chemicals in the database that are produced, used, released or present in California, and for which there is some information suggesting the chemicals may be carcinogenic. Prioritization of Batch #4 chemicals proceeded as described in the document entitled "Procedure for Prioritizing Candidate Chemicals for Consideration Under Proposition 65 by the State's Qualified Experts" (May 1997). Today marks the start of a 60-day public comment period on the draft data summaries for these 50 selected chemicals. A public workshop will be held on November 19, 2003 to receive verbal comments. Public comments received on the data summaries during the 60-day comment period will be reviewed and considered as part of the assignment of final priorities.

Prioritized chemicals with a final priority of "High" Carcinogenicity Concern are assigned to the Candidate List, from which chemicals will be chosen for the preparation of hazard identification documents. All chemicals not assigned a final "high" level of carcinogenic concern are assigned to Category II. Action is not anticipated on Category II chemicals until all high priority chemicals on the Candidate List with known or potential exposure have been evaluated.

It should be noted that (1) this prioritization process reflects a preliminary, rather than an in-depth review of carcinogenicity and exposure data, and, (2) the process is a continuous one; efforts to gather additional information on Category I and Category II chemicals are ongoing.

Name of Chemical	CAS No.	Level of Exposure Concern	Page
On Candidate List due to HIGH CARCINOGENICITY CONCERN			
4-Amino-N-(4,6-dimethyl-2-pyrimidinyl)benzene sulfonamide (sulfamethazine)	57-68-1	high	4
3,6-Dinitrobenzo[a]pyrene	128714-76-1	high	6
1,2-Epoxybutane	106-88-7	high	7
Methimazole	60-56-0	high	9
Molybdenum trioxide	1313-27-5	high	11
4-Nitrotoluene (*p*-nitrotoluene)	99-99-0	high	13
Nucleoside analogues: 3'-Azido-3'-deoxythymidine (AZT, zidovudine, N_3-ddT); 2',3'-Dideoxycytidine (ddC, zalcitabine); Stavudine (d4T); Trifluridine	 30516-87-1 7481-89-2 3056-17-5 70-00-8	 high high high high	15
Propoxur (Baygon)	114-26-1	high	17
Titanium dioxide	13463-67-7	high	19
1,2,4-Trichlorobenzene	120-82-1	high	21
Verapamil	52-53-9	high	23
2-Chloro-1,1,1-trifluoroethane	75-88-7	medium	25
4-Hydroxybenzenediazonium and its salts	19089-85-1	medium	27
4-Methylbenzenediazonium and its salts	57573-52-1	medium	29
Ciprofibrate	52214-84-3	low	31
Diallate	2303-16-4	n.i.c.	33
Diftalone	21626-89-1	n.i.c.	34
Category II (Not HIGH CARCINOGENICITY CONCERN)			
Acephate	30560-19-1	high	35
trans-Anethole	4180-23-8	high	37
Aspartame	22839-47-0	high	39
Chloroacetic acid	79-11-8	high	42
Chloromethane (methyl chloride)	74-87-3	high	44
Cholestyramine	11041-12-6	high	46
Clofentezine	74115-24-5	high	48
Cycloate	1134-23-2	high	49
3,4-Dihydrocoumarin	119-84-6	high	50
Flutamide	13311-84-7	high	52
Isoniazid	54-85-3	high	54

Name of Chemical	CAS No.	Level of Exposure Concern	Page
Levobunolol and its salts	47141-42-4	high	56
Methyl methacrylate	80-62-6	high	57
Mineral fibers, man-made; now referred to as Synthetic vitreous fibers: Rockwool (stonewool) Slagwool Continuous glass filaments	 ----- ----- -----	 high high high	59
Nicotine	54-11-5	high	61
3-Nitrofluoranthene	892-21-7	high	64
Orlistat	96829-58-2	high	67
Oxyfluorfen (Goal)	42874-03-3	high	68
Pyrimethamine (Daraprim)	58-14-0	high	69
Triethanolamine	102-71-6	high	71
Vitamin K (by intramuscular injection in neonates)	12001-79-5	high	73
Dimethipin (Harvade)	55290-64-7	medium	75
Mecoprop and its salts	7085-19-0	medium	77
Tralkoxydim	87820-88-0	medium	79
Triflusulfuron-methyl	126535-15-7	medium	80
Indolidan	100643-96-7	low	82
Isomazole and isomazole hydrochloride	86315-52-8; 87359-33-9	low	83
Acetoxymethylphenylnitrosamine	81943-37-5	n.i.c.	84
1-Benzoyl-2,6-dimethyl-4-nitrosopiperazine	61034-40-0	n.i.c.	85
INADEQUATE DATA to establish level of concern			
Pimozide	2062-78-4	high	86
POSTPONED		**Status**	
Chromium picolinate	14639-25-9	Awaiting completion of NTP bioassays	
Fusarium moniliforme (Fusarium verticillioides), toxins derived from	------	Candidate for administrative listing	
Sodium nitrite	7632-00-0	Review being completed	

n.i.c. = No Identified Concern

CARCINOGENICITY DATA SUMMARY: 4-AMINO-N-(4,6-DIMETHYL-2-PYRIMIDINYL)BENZENE SULFONAMIDE (SULFAMETHAZINE)

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 4-amino-N-(4,6-dimethyl-2-pyrimidinyl)benzene sulfonamide** (sulfamethazine; CAS No. 57-68-1), sufficient to place it on the candidate list. The high concern is based on multiple studies showing carcinogenic effects in animals.

Littlefield *et al.* (1989a) exposed groups of male and female Fischer 344/N rats to sulfamethazine in feed at concentrations of 0, 40, 600, 1200, or 2400 ppm for up to 24 months. The incidence of follicular cell adenocarcinomas of the thyroid was statistically significant by the Fisher's exact test in both sexes fed 2400 ppm and in females fed 1200 ppm (males: 0/180, 2/87, 0/90, 2/88, 2/83, 7/87 [$p < 0.001$]; females: 1/170, 0/90, 0/85, 0/84, 6/87 [$p < 0.01$], 6/88 [$p < 0.01$]). The incidence of thyroid follicular cell adenocarcinomas and adenomas combined was statistically significant by the Fisher's exact test in the 600, 1200 and 2400 ppm groups for both males and the 1200 ppm group in females, with the 2400 ppm female group of borderline significance (males: 0/180, 2/87, 0/90, 4/88 [$p < 0.05$], 4/83 [$p < 0.01$], 10/87 [$p < 0.001$]; females: 6/170, 0/90, 1/85, 4/84, 9/87 [$p < 0.05$], 8/88 [$p = 0.06$]).

Littlefield *et al.* (1989b) exposed groups of male and female B6C3F_1 mice to sulfamethazine at concentrations of 0, 300, 600, 1200, 2400 or 4800 ppm in feed for up to 24 months. Dosed animals developed follicular cell adenomas of the thyroid; increases were significant by the Fisher's exact test in the high dose group for both sexes (males: 2/184. 0/95, 1/92, 4/88, 4/94, 31/93 [$p < 0.001$]; females: 5/180, 1/91, 1/93, 0/95, 2/94, 23/89 [$p < 0.001$]). Three carcinomas of the thyroid were also observed (one male in the 2400 ppm group; one female at 600 ppm and one female at 4800 ppm). The incidence of hepatocellular carcinomas and adenomas was statistically significantly increased in high-dose females (10/184, 6/94, 11/94 [$p = 0.054$], 5/96, 7/96, 11/92 [$p < 0.05$]), with the Cochran-Armitage trend test of borderline significance ($p = 0.054$).

Sharma *et al*. (1989) reported in an abstract that genotoxicity assays in *Salmonella typhimurium* and *Escherichia coli* in the presence and absence of S9 were negative, as were a CHO/HGPRT mammalian gene mutation assay and an *in vivo* rat bone marrow chromosomal aberration assay. The International Agency for Research on Cancer (IARC, 2001) reported being "aware of data" showing that sulfamethazine induced sister chromatid exchanges in CHO cells in the absence but not the presence of metabolic activation. Pushpavathi *et al.* (1986) conducted a study of 31 factory workers exposed to a mixture of sulfa drugs, including sulfamethazine, and hydrogen chloride vapor. The frequency of chromosomal gaps and breaks in the exposed factory workers was significantly increased relative to controls.

IARC (2001) concluded that sulfamethazine is not genotoxic and that it would not be expected to be carcinogenic to humans exposed to concentrations below levels that could result in alterations in thyroid hormone homeostasis. IARC also concluded that rodents are "substantially more sensitive than humans to the development of thyroid tumors in response to thyroid hormone imbalance." Based on these conclusions, IARC classified sulfamethazine as Group 3 (not classifiable as to its carcinogenicity to humans), while also finding that there is sufficient evidence for the carcinogenicity of sulfamethazine in experimental animals.

There is a **HIGH** level of **concern over the extent of exposure** to sulfamethazine. Sulfamethazine is a sulfonamide drug that has been used to treat bacterial diseases in human and veterinary medicine and to promote growth in cattle, sheep, pigs, and poultry (IARC, 2001). Based on information from the Food and Drug Administration web site (www.fda.gov), sulfamethazine is approved for use in chickens, turkeys, cattle, and swine. A tolerance of 0.1 ppm in the meat of those animals has been established. Humans can be exposed via consumption of the meat of animals exposed to sulfamethazine. Sulfamethazine has been detected in wastewater (Kolpin *et al*., 2002).

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CARCINOGENICITY DATA SUMMARY: 3,6-DINITROBENZO[A]PYRENE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 3,6-dinitrobenzo[a]pyrene** (CAS No. 128714-76-1), sufficient to place it on the candidate list. The chemical induced malignant tumors in male rats, is genotoxic and is structurally similar to other carcinogenic compounds.

Male rats (Fischer 344/DuCrj) were injected subcutaneously one time at four dose levels of 3,6-dinitrobenzo[a]-pyrene in beeswax:tricaprilin and observed after 100 weeks (Tokiwa *et al.*, 1994; Horikawa *et al.*, 1998). Controls received only the vehicle. Injection site tumors, diagnosed histologically as malignant fibrous histiocytomas, were induced at doses of 40, 200 and 1000 μg/rat. The tumor latency period decreased with increasing dose.

3,6-Dinitrobenzo[a]pyrene exhibited direct mutagenic activity towards *Salmonella typhimurium* TA98 and related strains that are proficient in metabolic activity associated with oxygen-esterification (O-acetylation) (Sera *et al.*, 1991; Sera *et al.*, 1992; Tokiwa *et al.*, 1994).

3,6-Dinitrobenzo[a]pyrene is structurally similar to 1,6- and 1,8-dinitropyrene, which are on the Proposition 65 list of chemicals "known to the State" to cause cancer. 3,6-Dinitrobenzo[a]pyrene exhibited strong tumorigenic activity in the bioassay described above, although 1,6-dinitrobenzo[a]pyrene did not, even though each was applied at a similar dose level. Sera *et al*. (1991; 1992) reported the mutagenicity of 1,6-dinitrobenzo[a]pyrene towards *Salmonella typhimurium* TA98 was about one percent of that exhibited by a similar dose of 3,6-dinitrobenzo[a]-pyrene and, under conditions of O-acetylation, about five percent.

There is a **HIGH** level of **concern over the extent of exposure** to 3,6-dinitrobenzo[a]pyrene. 3,6-Dintrobenzo[a]-pyrene is expected to be ubiquitous in ambient air. It has been detected in airborne particulates (Sera *et al.*, 1991). The source is apparently emissions from diesel engines.

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CARCINOGENICITY DATA SUMMARY: 1,2-EPOXYBUTANE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 1,2-epoxybutane** (CAS No. 106-88-7), sufficient to place it on the candidate list, based on a positive cancer bioassay in male rats, supporting evidence from a bioassay in female rats, and extensive genotoxicity data demonstrating that 1,2-epoxybutane is a direct-acting alkylating agent.

The National Toxicology Program (NTP, 1988) found clear evidence of carcinogenic activity in male rats based on an increased incidence of nasal cavity papillary adenoma in the high dose group (0/50, 0/50, 7/50 [Fisher's exact test, $p < 0.01$]), and increased incidences of alveolar/bronchiolar carcinoma (0/50; 1/50; 4/49 [Fisher's exact test, $p = 0.056$]) and adenoma or carcinoma (combined) (0/50; 2/50; 5/49 [Fisher's exact test, $p < 0.05$]). NTP determined that there was equivocal evidence of carcinogenic activity in female rats, based on the occurrence of two nasal cavity papillary adenomas in the high dose group. Nonneoplastic lesions were observed in the nasal cavities of male and female mice, but carcinogenic effects were not found (NTP, 1988). A skin-painting study of 1,2-epoxybutane did not find evidence of tumors in female mice exposed three times per week for 77 weeks (IARC, 1989). The International Agency for Research on Cancer (IARC, 1989) reported on a combined exposure study, in which mice exposed via gavage to trichloroethylene containing 1,2-epoxybutane as a stabilizer developed squamous cell carcinoma of the forestomach. Animals exposed to trichloroethylene or corn oil alone did not develop tumors. IARC (1999) concluded that 1,2-epoxybutane is possibly carcinogenic to humans (Group 2B), based on limited evidence in animals and supporting data that demonstrate that the chemical is a direct-acting alkylating agent that is positive in a wide range of genotoxicity assays.

NTP (1988) and IARC (1999) have summarized the extensive data on the genotoxicity of 1,2-epoxybutane. The chemical was mutagenic in various strains of *Salmonella typhimurium*, with or without rat liver S9. 1,2-Epoxybutane showed mutagenic responses in the mouse lymphoma cell culture assay and in the L5178Y mouse lymphoma thymidine kinase assay. 1,2-Epoxybutane induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells with and without an exogenous metabolic system. The chromosomal aberration assay was considered "weakly positive" by NTP (1988), because the effects occurred at severely toxic doses. The chemical significantly increased sex-linked recessive lethal mutations and reciprocal translocations in *Drosophila melanogaster*, but not all reports were in agreement. NTP (1988) stated that the conflicting results in these studies were likely as a result of different doses and routes of administration. 1,2-Epoxybutane induced SOS repair activity in *Salmonella typhimurium* TA1525/pSK1002, produced differential killing zones in various *pol*- and *rec*-proficient and deficient strains of *Escherichia coli*, induced streptomycin-resistant mutants in *Klebsiella pneumoniae*, was mutagenic to *Escherichia coli* WP2 uvrA- (in one of two studies), induced forward mutations in *Schizosaccharomyces pombe* P1, induced mitotic recombination in *Saccharomyces cerevisiae* D3, was mutagenic in *Neurospora crassa*, and was marginally positive for induction of 6-thioguanine-resistant mutations in L5178Y cells. Morphological transformations were induced by 1,2-epoxybutane in Syrian hamster embryo cells and virally enhanced Fischer 344 rat embryo cells but were not observed in BALB/c 3T3 cells. The chemical did not induce unscheduled DNA synthesis in human fibroblast cells or in rat hepatocytes. NTP (1988) reported that 1,2-epoxybutane did not show effects in the available *in vivo* mammalian genotoxicity assays. Sperm abnormalities were not induced in male mice exposed to 1000 ppm 1,2-epoxybutane via inhalation (7 hours per day for 5 consecutive days). Dominant lethal mutations were not found in rats similarly exposed.

There is a **HIGH** level of **concern over the extent of exposure** to 1,2-epoxybutane. IARC (1999) reported that 1,2-epoxybutane is widely used as a stabilizer for chlorinated hydrocarbon solvents. NTP (1988) reported that eight million pounds of 1,2-epoxybutane were produced annually in the United States. A production volume of 10-50 million pounds was reported for 1,2-epoxybutane in the 1998 TSCA update (U.S. EPA, 1998).

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CARCINOGENICITY DATA SUMMARY: METHIMAZOLE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over methimazole** [1-methylimidazole-thiol; Tapazole®; Thiamazole; Mercazole; CAS No. 60-56-0], sufficient to place it on the candidate list. The chemical induced thyroid tumors in rats in multiple studies, and hepatomas in mice.

Significant increases in thyroid follicular adenomas and adenocarcinomas were observed in male and female rats treated with 0, 5, 30, or 180 ppm methimazole in the diet for two years (males/adenoma: 1/50, 1/25, 17/25 [$p < 0.001$], 5/25 [$p = 0.014$]; males/adenocarcinoma: 1/50, 0/25, 2/25, 3/25 [$p = 0.1$]; females/adenoma: 0/50, 0/25, 14/25 [$p < 0.001$], 12/25 [$p < 0.001$]; females/adenocarcinoma: 0/50, 0/25, 3/25 [$p = 0.034$], 2/25 [$p = 0.1$] [mortality not considered in denominator]) (Owen *et al.*, 1973). A drinking water study of short duration in male rats found increased thyroid follicular adenomas (Stoll *et al.,* 1980). A statistically significant increase in thyroid adenomas [$p = 0.002$] was reported in mice (results reported for males and females combined) treated with methimazole in drinking water on an "iodine-poor" diet (Jemec, 1977). Pulmonary metastases were observed in three of the thyroid tumor-bearing mice (Jemec, 1977; IARC, 2001). Increased incidences of hepatomas were observed in two sets of studies in mice (results reported for males and females combined) receiving methimazole in the drinking water (Jemec, 1970 [$p = 0.052$]; Jemec, 1977 [$p = 0.01$]).

Positive genotoxicity findings were reported for chromosomal aberrations in the C3H mouse mammary carcinoma cell line *in vitro* and sister chromatid exchange in C57BL6 mouse T lymphocytes *in vitro*, and inhibition of gap-junctional intercellular communication was observed in primary thyrocytes from Sprague-Dawley rats (reviewed by IARC, 2001). Negative genotoxicity findings have been reported in *Klebsiella pneumoniae* (forward mutation), Slc-ICR mouse bone-marrow cells (chromosomal aberrations), primary spermatocytes (chromosomal aberrations), and spermatogonia *in vivo* (chromosomal aberrations), Slc-ICR mouse bone-marrow cells *in vivo* (micronucleus formation), and male ICR mice *in vivo* (dominant lethal mutations) (reviewed by IARC, 2001). Methimazole achieves its clinical effect in the treatment of hyperthyroidism by inhibiting thyroid peroxidase, an essential enzyme in the synthesis of thyroid hormones (Capen, 1994).

IARC has reviewed epidemiological evidence for carcinogenicity of methimazole and found the evidence to be inadequate (IARC, 2001; citing cohort studies by Dobyns *et al.*, 1974, and Ron *et al.*, 1998, and case-control studies of Ron *et al.*, 1987, and Hallquist *et al.*, 1994). IARC has placed methimazole in Group 3, not classifiable as to carcinogenicity to humans based upon limited evidence in animals and inadequate evidence in humans (IARC, 2001).

In the section on Precautions of the FDA-approved label for Tapazole®, it is stated that: "Carcinogenesis, Mutagenesis, Impairment of Fertility - In a 2 year study, rats were given methimazole at doses of 0.5, 3, and 18 mg/kg/day. These doses were 0.3, 2, and 12 times the 15 mg/day maximum human maintenance dose (when calculated on the basis of surface area). Thyroid hyperplasia, adenoma, and carcinoma developed in rats at the two higher doses. The clinical significance of these findings is unclear" (Physicians Desk Reference, 2001).

There is a **HIGH** level of **concern over the extent of exposure** to methimazole. Methimazole is a thyrotropic pharmaceutical in common use for the treatment of hyperthyroidism (*e.g.*, Graves' disease). Therefore, high levels of exposure over a long time are expected for a subset of the population. Over four million people, mostly women, in the U.S. suffer from hyperthyroidism and are frequently treated with thionamides including methimazole (Thyroid Foundation of America, 2002).

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CARCINOGENICITY DATA SUMMARY: MOLYBDENUM TRIOXIDE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over molybdenum trioxide** (CAS No. 1313-27-5), sufficient for it to be placed on the candidate list. The high concern is based on molybdenum trioxide-induced pulmonary tumors in multiple studies and evidence of genotoxicity.

Male and female B6C3F$_1$ mice were exposed via inhalation (at 10, 30, or 100 mg/m^3 for 6 hr/day, 5 days/wk for 104 weeks). Male mice developed statistically significant increases in alveolar/bronchiolar carcinoma at all exposure levels (NTP, 1997; Chan *et al.*, 1998). Combined incidences of alveolar/bronchiolar adenoma/carcinoma were statistically significantly elevated in low and mid-dose male mice. Female mice developed significant increases in alveolar/bronchiolar adenoma in the mid- and high-dose groups. Alveolar/bronchiolar carcinoma was increased in high-dose female mice, though the increase was not statistically significant. The incidence of combined alveolar/bronchiolar adenoma/carcinoma was significantly elevated among high-dose female mice. NTP (1997) concluded that there was some evidence of carcinogenic activity in male and female B6C3F$_1$ mice. NTP (1997) similarly exposed F344/N rats. Alveolar/bronchiolar adenomas/carcinomas (combined) were increased in male rats with a positive trend, which NTP considered equivocal evidence of carcinogenic activity. No evidence of carcinogenic activity was observed in female rats.

Final mean body weights of high dose male and female rats and mice in the NTP studies were significantly lower than controls, however there were no clinical signs of toxicity, nor any effects on survival.

Stoner *et al.* (1976) observed a significant increase in the numbers of lung adenomas per mouse in Strain A/Strong mice exposed to molybdenum trioxide via intraperitoneal injection.

Molybdenum trioxide is water soluble and well absorbed after inhalation or oral exposure (NTP, 1997). It is excreted in the form of molybdate. NTP demonstrated increasing blood levels of molybdenum trioxide with increasing exposure.

NTP (1997) summarized cancer data on related compounds. Sodium molybdate has been shown to inhibit carcinogenesis induced by N-nitrososarcosine ethyl ester and N-nitroso-N-methylurea in animals. Sodium molybdate was also effective in preventing DNA damage by N-nitrosodiethylamine in rat liver. Molybdenum in the diet of rats inhibited carcinogenesis induced by N-methyl-N-benzylnitrosamine. Molybdenum dichloride inhibited the growth of Ehrlich ascites tumors in mice.

Five strains of *Salmonella typhimurium* tested for mutagenicity with molybdenum trioxide produced no positive results either with or without metabolic activation (NTP, 1997; Zeiger *et al.*, 1992). Sister chromatid exchange and chromosomal aberrations were not observed in CHO cells *in vitro* in response to molybdenum trioxide exposure (NTP, 1997). Molybdenum trioxide induced morphological transformations in a modified Syrian hamster embryo (SHE) cell transformation assay (Kearckaert *et al.*, 1996). Molybdenum trioxide was also positive in the Syrian hamster embryo (SHE) cell micronucleus assay (Gibson *et al.*, 1997). Titenko-Holland *et al.* (1998) reported on genotoxicity assays of ammonium molybdate and sodium molybdate and summarized results from other studies. The authors stated that these molybdenum salts were chosen for study because: 1) they are formed immediately upon addition of molybdenum trioxide to an aqueous buffered solution; 2) they represent the likely products of molybdenum trioxide upon interaction with body fluids; and 3) they are readily soluble and less toxic than other molybdenum compounds. Titenko-Holland *et al*. (1998) concluded that both salts were moderately positive at relatively high doses in the micronucleus assay in human lymphocytes *in vitro*, in the micronucleus assay in mouse bone marrow *in vivo*, and in the dominant lethal assay in mice. Other studies summarized by Titenko-Holland *et al.* (1998) found the following: 1) ammonium molybdate induced chromosome aberrations and sister chromatid exchanges in human lymphocytes *in vitro*; 2) lymphocytes from workers with more than 10 years of exposure to molybdenum showed increases in chromosome aberrations and sister chromatid exchanges; 3) molybdenum trioxide but not ammonium molybdate induced chromosome damage in mouse bone marrow; 4) ammonium molybdate in feed induced lethal mutations and decreased survival in *Drosophila*; and, 5) molybdenum compounds were not mutagenic in *Escherichia coli* or *Bacillus subtilis*.

There is a **HIGH** level of **concern over the extent of exposure** to molybdenum trioxide. Molybdenum trioxide is used as an additive to steel and other alloys, a chemical intermediate, an industrial catalyst, a pigment, a crop nutrient, a component of glass, ceramics and enamels, a flame retardant, and a chemical reagent. Annual production in the U.S. in 1979 was 110 million pounds (NTP, 1997). Since peaking in 1980, the production of molybdenum

has declined by 40% (USGS, 2002); current data on molybdenum trioxide were not located. NIOSH estimated that approximately 17,072 workers were potentially exposed to molybdenum trioxide during the years 1981 to 1983 (NTP, 1997). Releases of 435,269 pounds of molybdenum trioxide were reported in the California Toxic Release Inventory for 1999.

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CARCINOGENICITY DATA SUMMARY: 4-NITROTOLUENE (*P*-NITROTOULENE)

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 4-nitrotoluene** [*p*-nitrotoluene, methyl nitrobenzene; CAS No. 99-99-0], sufficient to place it on the candidate list. This is primarily due to increases in benign and malignant clitoral gland tumors in long-term studies in female F344/N rats fed diets containing 4-nitrotoluene (NTP, 2002a; Dunnick *et al*., 2003), positive clastogenicity findings, structural similarity to the more potent 2-nitrotoluene, and metabolism to 4-nitrobenzoic acid, a chemical that induces clitoral gland tumors in female rats.

There are no data on the carcinogenicity of 4-nitrotoluene in humans. A significantly increased incidence of clitoral gland adenoma or carcinoma (combined) was observed in female F344/N rats in the mid-dose group (8/50, 12/50, 20/50 ($p<0.008$), 8/49), which exceeded the historical control range. The incidence of clitoral gland neoplasms was not increased in the high-dose females. According to the National Toxicology Program (NTP, 2002a), this was possibly because of the lower body weights in this group, as similar reductions in the incidence of clitoral gland tumors have been observed in feed restriction studies. Exposure to 4-nitrobenzoic acid, a major metabolite of 4-nitrotoluene (36-45% of administered dose) in rats, increased the incidence of clitoral gland neoplasms in female F344/N rats (adenoma or carcinoma, combined: 4/50 [control], 14/49, 15/49, 15/50) (NTP, 1994). This provides further evidence that the chemical causes clitoral gland tumors in F344/N rats.

NTP (2002a) studies of 4-nitrotoluene in male rats and male mice add slightly to the evidence of carcinogenicity. The incidence of subcutaneous skin neoplasms (subcutaneous fibroma or fibrosarcoma (combined)) was increased in mid-dose male rats (1/50, 2/50, 9/50 ($p=0.01$), 1/50) and exceeded historical control ranges (NTP, 2002a). Subcutaneous skin tumors of the same types were also seen in male rats fed 2-nitrotoluene, but at much greater incidences than those associated with 4-nitrotoluene treatment. In male mice, the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was significantly greater in the high dose group than in controls (8/50, 14/50, 12/50, 19/50); incidence of alveolar epithelial hyperplasia in this group was also increased (NTP, 2002a). While the incidence of adenoma or carcinoma (combined) in the high dose group was within the range of historical controls fed the NTP-2000 diet, it exceeded the range in untreated controls from the larger NIH-07 historical database. No excess tumor incidences were observed in female mice (NTP, 2002a). NTP (2002a) considered the studies to provide some evidence of carcinogenicity in female rats, and equivocal evidence in male rats and male mice.

Results of genetic toxicology studies were reported by NTP (2002a). 4-Nitrotoluene was not mutagenic in any of several strains of *Salmonella typhimurium*, with or without metabolic activation enzymes (S9). A positive response was observed in the L5178Y mouse lymphoma cell assay in trials of 4-nitrotoluene with S9, and significantly increased sister chromatid exchange frequencies were observed in cultured Chinese hamster ovary cells with and without S9. Chromosomal aberrations were also induced in cultured Chinese hamster ovary cells treated with 4-nitrotoluene in the presence of S9; no increased aberrations were seen without S9. 4-Nitrotoluene did not induce a significant reproducible increase in the frequency of micronuclei in bone marrow polychromatic erythrocytes of male rats or male mice when administered by intraperitoneal injection.

The nitrotoluene isomer, 2-nitrotoluene, caused mesotheliomas, a rare tumor, in male rats after only 13 weeks of administration (NTP, 1992; Dunnick *et al*., 1994). The mesotheliomas were seen in the mid-dose group; mesothelial cell hyperplasia was seen in the high dose group. More recent studies of 2-nitrotoluene (NTP, 2002b) found neoplastic effects in multiple sites in male and female rats and mice. These include subcutaneous fibroma or fibrosarcoma (combined) in male rats (5/60, 47/60, 55/60, 59/60), the same site and type seen in studies of 4-nitrotoluene. The same site was also increased in female rats fed 2-nitrotoluene (3/60, 3/60, 21/60, 22/60) (NTP, 2002b). The major metabolite of 2-nitrotoluene is not *p*-nitrobenzoic acid, which may explain an absence of clitoral gland neoplasms in female rats fed 2-nitrotoluene. NTP (2002a) noted that in the NTP-conducted cancer bioassays 2-nitrotoluene was more carcinogenic than 4-nitrotoluene, as had been predicted from earlier studies showing that covalent binding of 2-nitrotoluene to total rat hepatic macromolecules is 3.5 times higher than that of 4-nitrotoluene. *In vitro* DNA binding studies have suggested that while *o*- and *p*-substituted arylamines both bind to DNA, the substitution in the *ortho* position yields a more stable DNA adduct (NTP, 2002a).

Based on a review done prior to completion of the NTP (2002a) studies, IARC (1996) classified 4-nitrotoluene (as part of a class, "Nitrotoluenes") in Group 3.

There is a **HIGH** level of **concern over the extent of exposure** to 4-nitrotoluene.
4-Nitrotoluene is used to synthesize agricultural and rubber chemicals, azo and sulfur dyes, and dyes for cotton,

wool, silk, leather, and paper (NTP, 2002a). Occupational exposure can occur in these industries, primarily through dermal and inhalation exposure; the recommended exposure limit is 2 ppm, with a skin notation, due to 4-nitrotoluene's potential to penetrate the skin in toxicologically significant quantities (ACGIH, 1991). An estimated 15 million pounds of 4-nitrotoluene are used annually in the United States (ACGIH, 1991). Production and use facilities are the major sources of potential environmental releases (Howard, 1989); 4-nitrotoluene has been detected in the air near and in water emitted from plants producing trinitrotoluene and various other compounds in the U.S. and other countries (IARC, 1996).

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CARCINOGENICITY DATA SUMMARY: NUCLEOSIDE ANALOGUES: 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT, ZIDOVUDINE, N_3-DDT), 2',3'-DIDEOXYCYTIDINE (DDC, ZALCITABINE), STAVUDINE (D4T), TRIFLURIDINE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 3'-azido-3'-deoxythymidine** [AZT, zidovudine, N_3-ddT; CAS No. 30516-87-1]**, 2', 3'-dideoxycytidine** [ddC, zalcitabine; CAS No. 7481-89-2]**, stavudine** [d4T**;** CAS No. 3056-17-5], and **trifluridine** [CAS No. 70-00-8], sufficient for these chemicals to be placed on the candidate list. The concern over these chemicals stems from observations of squamous cell carcinoma of the vagina among rodents exposed to 3'-azido-3'-deoxythymidine (AZT) as adults (Ayers *et al.*, 1996, 1997; NTP, 1999) and tumors at various sites following transplacental exposure of mice to AZT (Ayers *et al.*, 1997; Olivero *et al*., 1997; Diwan *et al*., 1999); lymphomas among male and female mice treated with 2',3'-dideoxycytidine (ddC) (Sanders *et al.*, 1995; Rao *et al.*, 1996); benign and malignant liver tumors in mice and rats and malignant urinary bladder tumors in male rats treated with stavudine (d4T) (PDR, 2000); and malignant cancers of the intestines, mammary gland and prostate in rodents treated with trifluridine (PDR, 2000); evidence of genotoxicity; and structural or functional similarities with the carcinogen azacytidine.

Nucleoside analogues are chemically-modified DNA or RNA bases, used primarily as anti-neoplastic and antiviral drugs. The chemical modifications can be on the nucleotide base or the sugar moiety or both. Nucleoside analogues function to inhibit DNA or RNA synthesis or to interfere with nucleic acid synthesis, resulting in alterations in DNA replication, gene function, and other vital cellular processes. Nucleoside analogues act by various mechanisms to inhibit DNA and RNA synthesis, including (1) incorporation into DNA or RNA, effectively terminating further strand synthesis, and (2) inhibition of DNA polymerase or related enzymes involved in DNA or RNA synthesis (Calabresi and Chabner, 1989; Douglas, 1989; Phillips *et al*., 1991; PDR, 2000; IARC, 2000).

Other nucleoside analogues exhibit evidence of carcinogenicity, based on increases in benign mammary, pancreatic and adrenal tumors in rats treated with rabavirin (PDR, 2000), observations of genotoxicity, primarily in mammalian cell assays, for brivudine, cytarabine, dideoxyadenosine, dideoxyinosine, fludarabine, gemcitabine, pentostatin, rabavirin, vidarabine (Simpson *et al.*, 1989; Calabresi and Chabner, 1989; Mamber *et al.*, 1990; Phillips *et al.*, 1991; PDR, 2000, Nabhan *et al*., 2001; IARC, 2000; CCRIS, 2002; GENE-TOX, 2002), and structural or functional similarities with the carcinogen azacytidine.

IARC classified AZT and 2', 3'-dideoxycytidine (zalcitabine, ddC) as possibly carcinogenic to humans (Group 2B) based on inadequate data in humans and sufficient evidence in animals (IARC, 2000).

IARC classified 2',3'-dideoxyinosine (ddI) and aciclovir as Group 3 (not classifiable as to its carcinogenicity) based on inadequate data in human and animals (IARC, 2000).

There is a **HIGH** level of **concern over the extent of exposure.** Many of the nucleoside analogues are approved drugs used to treat viral infections, cancer, or other diseases (PDR, 2000).

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CARCINOGENICITY DATA SUMMARY: PROPOXUR (BAYGON)

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over propoxur** [Baygon, methyl-O-isopropoxyphenyl methylcarbamate; CAS No. 114-26-1], sufficient to place it on the candidate list. This concern is based on the occurrence of bladder tumors (papilloma and carcinoma) in rats in multiple studies summarized by the California Department of Pesticide Regulation (CDPR, 1997) and the U.S. Environmental Protection Agency (U.S. EPA, 1997). Hyperplasia of the urinary bladder was also observed. In one set of studies in rats there was early onset of bladder tumors in both sexes. Uterine carcinoma was increased at the high dose in one rat study. Two-year bioassays in $B6C3F_1$ mice showed a significant increase in hepatocellular adenomas in the mid and high dose groups. Relevant non-neoplastic effects in the mouse studies include bladder epithelial hyperplasia in both sexes and ovarian nodules in females. Based on bladder tumors in rats and hepatocellular adenomas in $B6C3F_1$ mice, U.S. EPA (1997) classified propoxur as a Group B_2, probable human carcinogen. In one-year studies in NMRI mice and Syrian Golden hamsters, with the high dose similar to the high dose level used in the rat studies, no effect on the bladder was observed. These studies were not lifetime studies and were of insufficient length to detect late occurring tumors. Shukla *et al*. (1998) conducted initiation-promotion studies on propoxur and suggested that propoxur is tumorigenic and functions as a promoter. In 1989, the California Department of Food and Agriculture (CDFA) stated "that there is sufficient evidence to consider propoxur a potential carcinogen, and recommends that propoxur be added to the list of chemicals known to the State to cause cancer under Proposition 65" (CDFA, 1989).

Case-control studies have reported associations of propoxur exposure with lung cancer in licensed pest control workers in Florida (Pestori *et al*., 1994) and with infant acute leukemia following maternal exposure (Alexander *et al*., 2001).

While the mutagenicity studies submitted by the registrant for gene mutation, chromosomal aberration and DNA damage were all negative, a weakly mutagenic effect was observed with a metabolite of propoxur (CDPR, 1997). Negative results were also reported in gene mutation studies published in the open literature in *Salmonella typhimurium* and *Saccharomyces cerevisiae* (Gomez-Arroyo *et al*., 1995), *Escherichia coli* (PQ 37) (Vankat *et al*., 1995), *Bacillus subtilis* (Shirasu *et al*., 1976), and *Vibrio fischeri* (M169) (Gomez-Arroyo *et al*., 1995). A number of positive and negative results were reported in chromosomal aberration studies. With regard to *in vivo* chromosomal aberration studies, negative results were reported in bone marrow cells of male mice by Vasudev and Krishnamurthy (1994) and positive results by Agrawal (1999). Wei *et al*. (1997) reported that propoxur induced micronuclei *in vivo* in mice who received the pesticide orally or by intraperitoneal injection and *in vitro* in Chinese hamster ovary cells. Propoxur (or its nitroso derivative) induced sister chromatid exchange and micronuclei in human lymphocytes *in vitro* (Gonzalez *et al*., 1990). Gomez-Arroyo *et al.* (1995) reported propoxur-induced sister chromatid exchange, provided the pesticide was first incubated with a plant extract, presumably for metabolic activation. Wang *et al.* (1998) observed genotoxicity in hamster lung fibroblasts (V79) and rat tracheal epithelial cells with the chemically produced N-nitroso derivative of propoxur, but not with the unaltered propoxur.

There is a **HIGH** level of **concern over the extent of exposure**. Propoxur is a carbamate pesticide used by homeowners and pest control operators to control roaches and other pests in and around residences, commercial and industrial buildings as well as in food handling establishments. It is present in about 100 products actively registered in California. CDPR (2002) reported that 611 pounds were used in agricultural and commercial applications in California in 2001. U.S. EPA (1997) estimated total use of propoxur to range between 170,000 to 400,000 pounds per year, with 150,000 to 350,000 pounds of that being used by consumers.

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CARCINOGENICITY DATA SUMMARY: TITANIUM DIOXIDE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over titanium dioxide** (CAS No. 13463-67-7), sufficient to place it on the candidate list. The high concern is based on the induction of lung tumors in rats in multiple studies. An occupational epidemiology study by Chen and Fayerweather (1988) was described by the International Agency for Research on Cancer (IARC, 1989) as inconclusive. Subsequent to the IARC review, one epidemiology study reported a weak association of titanium dioxide exposure and bladder cancer (Siemiatycki *et al*., 1994). A population based case-control study by Boffetta *et al*. (2001), an update of Siemiatycki *et al.* (1994), found no significant increase in lung cancer among workers occupationally exposed to titanium dioxide (odds ratio 0.9, 95% CI 0.5—1.5).

Inhalation studies in male and female CD-1 rats by Lee *et al*. (1985), also reported in Trochimowicz *et al*. (1988), demonstrated increases in bronchiolar/alveolar and squamous cell tumors of the lung at the highest dose tested, 250 mg/m^3 for six hr/day, five days/week. Both benign and malignant tumors were found, with malignant tumors more frequent in females than in males (females, benign tumors: 13/74 in 250 mg/m^3 dose group *vs*. 0/77 in controls [$p<0.001$], malignant tumors: 13/74 in 250 mg/m^3 dose group *vs*. 0/77 in controls [$p<0.001$]; males, benign tumors: 12/77 in 250 mg/m^3 dose group *vs*. 2/79 in controls [$p=0.004$], malignant tumors: 1/77 in 250 mg/m^3 dose group *vs*. 0/79 in controls [$p=0.49$]). Significant increases in benign and malignant bronchiolar/alveolar and squamous cell tumors ($p<0.001$) were observed in female Wistar rats treated by inhalation with 10.4 mg/m^3 titanium dioxide, 18 hr/day, five days/week for 24 months (benign tumors: 24/100, malignant tumors: 16/100; no lung tumors were observed in controls) (Dungworth *et al*., 1994). No treatment related increases in tumors were observed in inhalation studies of rats exposed to titanium dioxide concentrations of 16 mg/m^3 six hr/day, five days/week for 12 weeks (Thyssen *et al*., 1978) or 5 mg/m^3 six hr/day, five days/week for two years (Muhle *et al.,* 1991). A study of intratracheal administration of titanium dioxide in male hamsters by Mohr *et al*. (1984) reported two sarcomas of the thorax in 135 animals.

Studies of titanium dioxide administered in feed or by intraperitoneal injection did not observe treatment-related tumors in mice or rats. No treatment-related tumors were observed in studies conducted by the National Cancer Institute (NCI), where groups of 50 male and female $B6C3F_1$ mice and 50 male and female Fischer 344 rats were administered titanium dioxide at 0, 25,000 ppm or 50,000 ppm in the diet for 103 weeks (NCI, 1979). Based on these studies, NCI reported that titanium dioxide was not carcinogenic by the oral route in mice or rats (NCI, 1979). Studies by Bernard *et al*. (1990) found no evidence of treatment-related tumors in male and female Fischer 344 rats fed diets containing up to 5.0% titanium dioxide-coated mica particles for 130 weeks. Bischoff and Bryson (1982) found no increased incidence of tumors in male Marsh-Buffalo mice administered 25 mg of titanium dioxide by intraperitoneal injection. In rats, Maltoni *et al.* (1982) reported no increased incidence of tumors in animals administered 30 mg titanium dioxide by intraperitoneal injection.

No reports of genotoxicity studies in humans *in vivo* were identified and no chromosomal aberrations were detected in cultured human lymphocytes treated with titanium dioxide (IARC, 1989). Micronuclei were observed in the bone marrow cells of mice given intraperitoneal injections of titanium dioxide at 25, 50, or 100% of the maximum tolerated dose (Shelby *et al*., 1993). No chromosomal aberrations or sister chromatid exchanges were induced in Chinese hamster ovary cells (Ivett *et al*., 1989). Gallagher *et al*. (1994) observed no increase in DNA adducts in lung tissue from titanium dioxide exposed female Wistar rats compared with controls.

In its 1989 review, IARC concluded that titanium dioxide was not classifiable as to its carcinogenicity to humans (Group 3), based on inadequate evidence in humans and limited evidence in experimental animals (IARC, 1989).

There is a **HIGH** level of **concern over the extent of exposure** to titanium dioxide. Titanium dioxide is a white crystalline solid used as a whitening agent in paints, varnishes, paper, plastics, ceramics, rubber, and printing ink (ACGIH, 1991). It is used as an additive in polymer production, electronic component production and in the production of alcohol fuels. In addition, it is used in the formulation of topical and oral pharmaceuticals and as a colorant in foods (ACGIH, 1991). Workers in many occupations are exposed to titanium dioxide particles, primarily when titanium dioxide is ground into powder and added to various formulations. Because of its widespread use as a food additive, the general population is exposed through ingestion.

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CARCINOGENICITY DATA SUMMARY: 1,2,4-TRICHLOROBENZENE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 1,2,4-trichlorobenzene** (CAS No. 120-82-1), sufficient for it to be placed on the candidate list. The level of concern is based on unusually high treatment-related increases in hepatocellular adenoma and carcinoma in male and female mice.

The results of a set of 104-week dietary carcinogenicity studies in male and female mice conducted by Hazleton were submitted by the Chemical Manufacturers' Association (CMA) to the U.S. Environmental Protection Agency (U.S. EPA, 1993a; 1993b). The Office of Environmental Health Hazard Assessment (OEHHA, 1999) summarized the key findings of these studies. Male and female $B6C3F_1$ mice exposed to 1,2,4-trichlorobenzene developed statistically significant increases in hepatocellular adenoma and carcinoma. The incidence of hepatocellular carcinoma was greater than 50% in both sexes treated with 700 mg/kg-day. In the high-dose groups (3200 mg/kg-day), the incidence of hepatocellular carcinoma was 100% in male mice and 92% in female mice. Survival was significantly reduced in high dose animals; this was attributed to deaths due to the hepatocellular neoplasms. Mean body weights were also reduced in high dose animals relative to controls.

In a similar set of studies in male and female rats, no carcinogenic effects were observed. A skin painting study in mice by Yamamoto *et al*. (1982; as cited by U.S. EPA, 1991) did not report any treatment-related increases in tumors. U.S. EPA (1991) discussed a number of limitations of the study: inadequate reporting, dosing occurred only twice weekly, female mice were housed as a group, and survival was poor (80% of control mice and 90% of treated mice died before the end of the study).

1,2,4-Trichlorobenzene has been tested in a number of genotoxicity assays, the results of which were summarized by OEHHA (1999). No effects were reported in assays with seven strains *Salmonella typhimurium* or in *Escherichia coli* with or without metabolic activation, in a CHO chromosomal aberration study with or without metabolic activation, or in rat hepatocyte primary culture DNA repair tests. 1,2,4-Trichlorobenzene was found to induce cellular transformation in adult rat liver epithelial cells. A dose-related increase was observed in the number of micronucleated cells in bone marrow in eight-week old mice exposed to 1,2,4-trichlorobenzene via intraperitoneal injection.

U.S. EPA (1991) classified 1,2,4-trichlorobenzene as a Group D (not classifiable as to human carcinogenicity), but this classification did not consider the study submitted by CMA. 1,2,4-Trichlorobenzene is designated as a Toxic Air Contaminant in California.

There is a **HIGH** level of **concern over the extent of exposure** to 1,2,4-trichlorobenzene. 1,2,4-Trichlorobenzene is used as a solvent and herbicide. It is registered in California, though no active products were identified (California Department of Pesticide Regulation, 2003). A production volume of between 10 and 50 million pounds was reported in the Toxic Substances Control Act 1998 Inventory Update (U.S. EPA, 1998).

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CARCINOGENICITY DATA SUMMARY: VERAPAMIL

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over verapamil** [Calan ®; benzeneacetonitrile, alpha-(3-((2-(3,4-diethoxyphenyl)ethyl)methylamino)propyl)-3,4-dimethoxy-alpha-(1-methylethyl); CAS No. 52-53-9], sufficient to place it on the candidate list. Epidemiological studies have consistently observed increased cancer risk in patients using verapamil.

The most recently published study, the Rotterdam study, a prospective population-based cohort study (n=3204) in The Netherlands, collected information on cumulative dose, exposures to other drugs, and other potential confounders (Beiderbeck-Noll *et al.,* 2003). These authors found a significant increase in cancer risk for verapamil use after adjustment for all known independent risk factors (RR=2.0, 95% CI=1.01-3.9, n=9 cancers among verapamil users); this increase was dose-dependent (low dose, RR=1.7, 95% CI=0.7-4.2; medium dose, RR=2.7, 95% CI=1.02-7.4; high dose, no cases). A significantly increased risk of cancer was found for cumulative use of verapamil (≤2 years, RR=1.5, 95% CI=0.9-2.5; >2 years, RR=2.4, 95% CI=1.2-5.0) . Risk for cancers of the lymphatic and hematopoietic organs was statistically significantly increased (RR=7.84, 95% CI=1.66-37.0) (Beiderbeck-Noll *et al.,* 2003).

Most available epidemiological studies are limited not only by small numbers of verapamil-exposed patients but also by lack of information on cumulative dose or control for exposures to other drugs. In studies which had specific analyses of patients taking verapamil, increased risks were seen in several studies (Hardell *et al*., 1996: RR=22, 95% CI=2.4-480, n=10 colon cancer patients; Pahor *et al*., 1996: Hazard ratio=2.49, 95% CI=1.54-4.01, p=0.0002 in an adjusted model for all cancers, n=118 users of verapamil; Sajadieh *et al*., 1999: SIR=3.9, 95% CI=1.3-9.1, based on five cases of lung cancer in women, n=26 women using verapamil).

Additional studies of cancer in patients using calcium channel blockers have grouped together all of the drugs with this activity (*e.g.*, Michels *et al*., 1998; Sorensen *et al*., 2000), and some but not all found indications of increased cancer risk at one or more sites. For calcium channel blockers as a group, evidence of a dose-response effect was seen in one study (Pahor *et al*., 1996).

A biologically plausible mechanism of carcinogenicity has been proposed for calcium channel blockers such as verapamil: for cells in which a sustained rise of cystolic calcium initiates the apoptotic process, the use of calcium channel blockers may act as a tumor promoter by interfering with the programmed death of DNA-damaged cells. A recent review (Mason, 1999) of the role of calcium channel blockers on apoptosis found contradictory evidence, with many studies showing inhibition of apoptosis, and many others showing promotion of apoptosis. No treatment-related increase in tumors was reported in one set of studies in male and female rats exposed to verapamil in the diet for two years at doses up to 12 times the maximum recommended human daily dose of 9.6 mg/kg-day) (PDR, 2000). Verapamil was not mutagenic in the Ames test in five test strains at 3 mg per plate with and without metabolic activation (PDR, 2000). However, studies have identified co-mutagenic activity (Ferguson and Bagley, 1988; Scheid *et al*., 1991) and co-clastogenic activity (Nesterova *et al*., 1999) of verapamil.

There is a **HIGH** level of **concern over the extent of exposure** to verapamil. Verapamil is a calcium-channel blocker used to treat hypertension and other cardiovascular problems. Millions of patients are currently taking calcium antagonists including verapamil (Pahor and Furberg, 1998).

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CARCINOGENICITY DATA SUMMARY: 2-CHLORO-1,1,1-TRIFLUOROETHANE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 2-chloro-1,1,1-trifluoroethane** [hydrochlorofluorocarbon-133a, HCFC-133a, CAS No. 75-88-7], sufficient to place it on the candidate list. This concern is primarily based on unusually high occurrences of malignant uterine cancers and testicular interstitial cell tumors in treated Alph/Ap (Wistar derived) rats (Longstaff *et al.,* 1984). Incidences of uterine cancers were 1/104 in controls and 15/35 in treated female rats ($p < 1x10^{-9}$), and of testicular cancers were 16/104 in controls and 29/36 in treated male rats ($p < 1x10^{-11}$). The International Agency for Research on Cancer (IARC, 1986 and 1999) classified 2-chloro-1,1,1-trifluoroethane in Group 3, based on inadequate data in humans and limited data in experimental animals.

2-Chloro-1,1,1-trifluoroethane did not cause mutations in the reverse mutation assay in *Salmonella*, was negative in a cell transformation assay in hamster kidney fibroblasts *in vitro*, and did not induce chromosomal aberrations in rat bone-marrow cells *in vivo* (Longstaff *et al.*, 1984; IARC, 1986; 1999).

Compounds structurally similar to 2-chloro-1,1,1-trifluoroethane caused tumors at estrogen-responsive sites. Chloroethane induced a high incidence of uterine carcinomas of endometrial origin in female mice (control, 0/49; exposed, 43/50) (NTP, 1989a). Bromoethane induced squamous cell carcinomas of the uterus in mice (NTP, 1989b). In this regard, metabolic dehalogenation of 2-chloro-1,1,1-trifluoroethane is expected to be similar to chloroethane and bromoethane (Salmon *et al.*, 1981; 1985). Other halogenated alkanes have induced uterine tumors in mice: 1,1-dichloroethane (NTP, 1978a), 1,2-dichloroethane (NTP, 1978b), and 1,2,3-trichloropropane (NTP, 1993). Dibromochloropropane (NTP, 1978c), 1,2-dibromoethane (NTP, 1982), 1,2-dichloroethane (NTP, 1978a) and 1,2,3-trichloropropane (NTP, 1993) induced mammary gland tumors in rodents.

There is a **MEDIUM** level of **concern over the extent of exposure** to 2-chloro-1,1,1-trifluoroethane. 2-Chloro-1,1,1-trifluoroethane is used as a chemical intermediate in the production of the anesthetic halothane (IARC, 1999). 23,450 pounds were reported as on- and off-site releases to the U.S. EPA 1997 Toxic Release Inventory (U.S. EPA, 1999). Additional exposures occur as 2-chloro-1,1,1-trifluoroethane is an impurity and metabolite of halothane (Baker and Van Dyke, 1984).

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CARCINOGENICITY DATA SUMMARY: 4-HYDROXYBENZENEDIAZONIUM AND ITS SALTS

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 4-hydroxybenzenediazonium** (HBD; CAS No. 19089-85-1) **and its salts** (*e.g.*, 4-hydroxybenzenediazonium sulfate (HBD sulfate; CAS No. 41279-80-5)) based upon subcutaneous injection studies showing an increase in subcutaneous and skin tumors in male and female mice and strong structural similarity to other compounds which cause a similar spectrum of tumors.

Male and female Swiss mice (n = 50/sex) injected subcutaneously weekly 36 times with 2 micrograms HBD sulfate showed an increase in tumors of the subcutis relative to untreated controls (Toth *et al.*, 1989a). Eleven female mice treated with HBD sulfate developed tumors which were classified as fibromas (1/50), fibrosarcomas (8/50), and rhabdomyosarcomas (2/50), whereas only one control female mice developed a fibrosarcoma. Likewise, 11 male mice developed fibrosarcomas (9/50), a rhabdomyosarcomas (1/50), and a myxosarcomas (1/50), compared to four fibrosarcomas in the untreated male mice. The increase in total subcutaneous tumor incidence over control incidence is statistically significant in both male and female mice. Mice (50/sex) receiving two weekly subcutaneous injections of 10 micrograms HBD sulfate showed no increase in tumor incidence.

Several other structurally-related compounds have been examined for carcinogenicity in a series of studies by the same authors. These compounds include 4-methylbenzenediazonium (MBD) sulfate, 4-hydroxymethylbenzene-diazonium (HMBD) sulfate and tetrafluoroborate, and benzenediazonium sulfate.

Among mice (50/sex/group) injected subcutaneously weekly 16 times with MBD sulfate, then observed for life, significant increases in tumors of the subcutis (males and females) and skin (females only) were observed (Toth *et al.*, 1989b, previously reported in an abstract: Lawson *et al.*, 1988). Among mice similarly injected subcutaneously weekly 19 times with MBD, only a marginal increase in tumors of the subcutis was observed. The tumors seen in these studies were classified as fibromas, fibrosarcomas, myxosarcomas, fibromyxosarcomas, rhabdomyosarcomas, and angiosarcomas of the subcutis and squamous cell papillomas and carcinomas of the skin.

Male and female Swiss mice injected subcutaneously weekly 26 times with HMBD sulfate developed a significant increase in subcutaneous tumors (males and females) and skin tumors (females only) (Toth, 1987; only author's abstract available). The subcutaneous tumors were identified as fibrosarcomas, rhabdomyosarcomas, and angio-sarcomas. The skin tumors were identified as squamous cell papillomas and carcinomas.

Swiss mice (n = 50/sex) injected subcutaneously weekly 26 times with HMBD tetrafluoroborate developed increases in the incidence of subcutaneous tumors (20% *vs.* 6% controls) and skin tumors (12% *vs.* 0% controls) (Toth *et al.*, 1981). The subcutaneous tumors were classified as fibroma, fibrosarcomas, rhabdomyosarcomas, and an angiosarcoma, and the skin tumors as squamous cell papillomas and carcinomas.

Swiss mice (n = 50/sex) treated with a single intragastric dose of HMBD tetrafluoroborate developed an increase in the incidence of polyploid adenomas and adenocarcinomas of the forestomach (30% treated females *vs.* 2% vehicle controls; 32% treated males *vs.* 0% vehicle controls) (Toth *et al.*, 1982).

Male and female Swiss mice treated with weekly subcutaneous injections of benzenediazonium sulfate for 26 weeks developed an increase in the incidence of tumors of the subcutis relative to controls (Toth *et al.*, 1998). The tumors were described as fibromas, rhabdomyosarcomas, and osteosarcomas of the subcutis. Another study in which treatment occurred for 10 weeks resulted in a non-statistically significant increase in tumors of the subcutis alone (Toth *et al.*, 1999). Also reported in the latter publication were oral gavage studies in which Swiss mice were administered weekly doses of benzenediazonium sulfate for 52 weeks which showed an increase in the incidence of lung adenomas and adenocarcinomas among both male and female mice.

Aqueous extracts of baked and raw *Agaricus bisporus*, a mushroom that has been demonstrated by Ross *et al*. (1982) to contain the HMBD ion, were found to be mutagenic in *Salmonella typhimurium* strains TA1535 with and without metabolic activation, and in strain TA1537 (Toth *et al.*, 1992). HMBD has been implicated in causing DNA strand breaks (Hiramoto *et al.*, 1995).

There is a **MEDIUM** level of **concern over the extent of exposure** to HBD and its salts. HBD is likely to be present in edible mushrooms and has been found in *Agaricus xanthodermus*, a non-cultivable inedible mushroom closely related to the commonly cultivated and consumed mushroom *Agaricus bisporus* (Dornberger *et al.*, 1986).

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CARCINOGENICITY DATA SUMMARY: 4-METHYLBENZENEDIAZONIUM AND ITS SALTS

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over 4-methylbenzenediazonium** (MBD; CAS No. 57573-52-1) **and its salts** (*e.g.*, 4-methylbenzenediazonium sulfate (MBD sulfate; *p*-toluenediazonium sulfate; CAS No. 32066-79-8)) based upon subcutaneous injection studies showing a significant increase in subcutaneous and skin tumors in male and female mice and strong structural similarity to other compounds which cause a similar spectrum of tumors.

Among mice (50/sex/group) injected subcutaneously weekly 16 times with MBD sulfate, then observed for life, significant increases in tumors of the subcutis (males and females) and skin (females only) were observed (Toth *et al.*, 1989b, previously reported in an abstract: Lawson *et al.*, 1988). Among mice similarly injected subcutaneously weekly 19 times with MBD, only a marginally significant increase in tumors of the subcutis was observed (Toth *et al.*, 1989b). The tumors seen in these studies were classified as fibromas, fibrosarcomas, myxosarcomas, fibromyxosarcomas, rhabdomyosarcomas, and angiosarcomas of the subcutis and squamous cell papillomas and carcinomas of the skin.

Several other structurally-related compounds have been examined for carcinogenicity in a series of studies by the same authors. These compounds include 4-hydroxybenzenediazonium (HBD) sulfate, 4-hydroxymethylbenzenediazonium (HMBD) sulfate and tetrafluoroborate, and benzenediazonium sulfate.

Male and female Swiss mice injected subcutaneously weekly 19 times with HBD sulfate showed an increase in tumors of the subcutis (Toth *et al.*, 1989a). The tumors were classified as fibromas, fibrosarcomas, myxosarcomas and rhabdomyosarcomas.

Male and female Swiss mice injected subcutaneously weekly 26 times with HMBD sulfate developed a significant increase in subcutaneous tumors (males and females) and skin tumors (females only) (Toth, 1987; only author's abstract available). The subcutaneous tumors were identified as fibrosarcomas, rhabdomyosarcomas, and angiosarcomas. The skin tumors were identified as squamous cell papillomas and carcinomas.

Swiss mice (n = 50/sex) injected subcutaneously weekly 26 times with HMBD tetrafluoroborate developed increases in the incidence of subcutaneous tumors (20% *vs.* 6% controls) and skin tumors (12% *vs.* 0% controls) (Toth *et al.*, 1981). The subcutaneous tumors were classified as fibroma, fibrosarcomas, rhabdomyosarcomas, and an angiosarcoma, and the skin tumors as squamous cell papillomas and carcinomas.

Swiss mice (n = 50/sex) treated with a single intragastric dose of HMBD tetrafluoroborate developed an increase in the incidence of polyploid adenomas and adenocarcinomas of the forestomach (30% treated females *vs.* 2% vehicle controls; 32% treated males *vs.* 0% vehicle controls) (Toth *et al.*, 1982).

Male and female Swiss mice treated with weekly subcutaneous injections of benzenediazonium sulfate for 26 weeks developed an increase in the incidence of tumors of the subcutis relative to controls (Toth *et al.*, 1998). The tumors were described as fibromas, rhabdomyosarcomas, and osteosarcomas. Another study in which treatment occurred for 10 weeks resulted in a non-statistically significant increase in tumors of the subcutis alone (Toth *et al.*, 1999). Also reported in the latter publication were oral gavage studies in which Swiss mice were administered weekly doses of benzenediazonium sulfate for 52 weeks which showed an increase in the incidence of lung adenomas and adenocarcinomas among both male and female mice.

Aqueous extracts of baked and raw *Agaricus bisporus*, a mushroom that has been demonstrated by Ross *et al*. (1982) to contain the HMBD ion, were found to be mutagenic in *Salmonella typhimurium* strains TA1535 with and without metabolic activation, and in strain TA1537 (Toth *et al.*, 1992). HMBD has been implicated in causing DNA strand breaks (Hiramoto *et al.*, 1995).

While the mechanism by which tumors develop following exposure to arenediazonium ions is unknown, studies of 4-methylbenzenediazonium ion have shown that it activates the transcription factor AP-1, possibly through the generation of reactive oxygen species (Gannett *et al.*, 2000; Powell and Gannett, 2002).

There is a **MEDIUM** level of **concern over the extent of exposure** to MBD and its salts, although direct exposure information for MBD was not readily discerned from the available literature. MBD has been speculated to be converted from HMBD. HMBD is a fungal metabolite of agaritine, a naturally occurring substance found in

Agaricus species. HMBD ion has been detected in the commonly cultivated and consumed mushroom *Agaricus bisporus* at levels near 0.6 ppm (Ross *et al.*, 1982).

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CARCINOGENICITY DATA SUMMARY: CIPROFIBRATE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over ciprofibrate** (Modalim® (UK); 2-[4-(2,2-dichlorocyclopropyl)phenoxyl]-2-methyl propanoic acid; CAS No. 52214-84-3), sufficient for it to be placed on the candidate list. In multiple studies this compound caused liver tumors in rats and mice.

Multiple long-term studies in male Fischer rats in which ciprofibrate was administered in feed have produced a high incidence of liver tumors among treated animals (Rao *et al.*, 1984; Rao *et al.*, 1986; Milano *et al.,* 1987; Rao and Subbarao, 1997; Rao and Subbarao, 1999; all as described in NIH, 1986). In a single study of male and female Fischer rats treated with ciprofibrate in feed, a significant increase in stomach carcinoid tumors was observed in males (Spencer *et al.,* 1989; as described in NIH, 1991). In a long-term feeding study in male C57BL/6N mice, ciprofibrate induced liver tumors (Rao *et al.,* 1988; as described in NIH, 1989). Oral gavage studies in male and female *Callithrix jacciux* marmosets (n = 4/sex) treated with ciprofibrate for 155 weeks produced no evidence of tumors at 155 weeks (Graham *et al.* 1994; as described in NIH, 1996)

Ciprofibrate did not induce unscheduled DNA synthesis in the absence of metabolic activation in cultured hepatocytes (Glauert *et al.*, 1984; Selden *et al.*, 1994, each described CCRIS, 1995). Ciprofibrate did not induce mutations in *Salmonella typhimurium* (TA102, TA104, TA98) in the absence or presence of metabolic activation (Glauert *et al.*, 1984). Numerous studies have demonstrated that ciprofibrate belongs to the class of compounds called peroxisome proliferators which cause liver cancer by a mechanism involving a cellular receptor called peroxisome proliferator activated receptor-alpha (PPAR-α).

There is a **LOW** level of **concern over the extent of exposure** to ciprofibrate. Ciprofibrate is a blood lipid lowering drug, but is not approved by the U.S. Food and Drug Administration for use in the U.S. Ciprofibrate appears to be in use in other countries, although its use in France was restricted in 1995 due to the potential for rhabdomyolysis among patients taking the drug. Ciprofibrate is in clinical trials in countries other that the U.S., particularly in combination with statin drugs.

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CARCINOGENICITY DATA SUMMARY: DIALLATE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenic concern over diallate** (CAS No. 2303-16-4), sufficient for it to be placed on the candidate list. The high concern is based on carcinogenic effects in multiple experiments. Human carcinogenicity data are not available.

Innes *et al.* (1969; as cited by U.S. Environmental Protection Agency [U.S. EPA], 1983) found significant increases in hepatomas in male and female mice and pulmonary tumors in male mice exposed to diallate via gavage and diet. BRL (1968; as cited by U.S. EPA, 1983) observed a significantly increased incidence of reticulum cell sarcoma in male mice exposed to a single subcutaneous injection of diallate. Monsanto (U.S. EPA, 1982) observed significant increases in dermal melanoma in Syrian hamsters exposed via diet. Darnis (1980; as cited by U.S. EPA, 1983) reportedly found increases in liver tumors in mice and rats after oral or subcutaneous administration, but only an abstract of the study was available. U.S. EPA (1982) mentions a study by Keplinger *et al.* (1976) in Albino rats as being positive, but no details are provided. U.S. EPA (1983) cites two studies in rats as finding no oncogenic effects.

U.S. EPA (1983) considers diallate a mutagen requiring metabolic activation. There are numerous positive genotoxicity studies in a wide range of assays (*e.g.*, Sandhu *et al.*, 1984).

U.S. EPA tentatively classified diallate as a B2 carcinogen, but that classification was never finalized. The International Agency for Research on Cancer (IARC, 1983) placed diallate in Group 3 based on inadequate evidence in humans and limited evidence in animals, but did not have all of the bioassay data reviewed by U.S. EPA.

There is **NO IDENTIFIED CONCERN over the extent of exposure** to diallate in California. Diallate is not registered for use in California and the U.S. EPA revoked all tolerances for diallate in 1994.

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CARCINOGENICITY DATA SUMMARY: DIFTALONE

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH level of carcinogenicity concern over diftalone** [phthalazino-(2,3-b)phthalazine-5,12(7H,14H)-dione; CAS No. 21626-89-1], sufficient to place it on the candidate list. Clear carcinogenic effects were observed in both sexes of BALB/cLacDp mice. Carcinogenic effects were also reported in rats but the studies were not available to OEHHA.

No human data on long-term effects of exposure to diftalone are available. Regarding animal bioassay data, groups of 39-50 male and female BALB/cLacDp mice were fed a standard pellet diet containing 0, 300, 600 or 1200 ppm diftalone for 80 weeks (starting at eight weeks of age) then fed a control diet until 126-128 weeks of age when sacrificed (Della Porta *et al*., 1984). In the highest dose groups, angiosarcomas and benign and malignant hepatocellular tumors were observed in females and angiomas and angiosarcomas were observed in treated males. There was an increased incidence of lung tumors, but a positive trend was only significant in the females. Liver toxicity was also observed in the highest dose group, with a slight decrease in body weight observed in all treated females. Della Porta *et al.* (1984) reported that hepatocellular carcinomas were observed in male and female rats and mice in studies conducted by Dow Chemical, but details of these studies were not presented.

Diftalone was not mutagenic in the presence or absence of metabolic activation in *Salmonella typhimurium* strains TA 98, TA 100, or TA 102 (Della Porta *et al*., 1984). No information on the genotoxicity of diftalone in mammalian assay systems was identified.

There is **NO IDENTIFIED CONCERN over the extent of exposure** to diftalone. Diftalone is a non-steroidal anti-inflammatory drug that was explored for use in the treatment of rheumatoid arthritis. However, the U.S. Food and Drug Administration (2003) does not show any record of current use and the drug does not appear in the Physicians' Desk Reference (2001).

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CARCINOGENICITY DATA SUMMARY: ACEPHATE

Preliminary evaluation of carcinogenicity and exposure data

Acephate (O, S-dimethyl acetylphosphoramidothioate; CAS No. 30560-19-1) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, although there is some carcinogenic concern based on the finding of liver tumors in female mice.

The U.S. Environmental Protection Agency (U.S. EPA, 1999) summarized the data on the carcinogenicity of acephate. Acephate was tested in two-year feeding studies in Charles River CD1 mice (Spicer *et al.*, 1982; Geil, 1981; Leary, 1981; Glickman, 1983; all as cited by U.S. EPA, 1999). There was a statistically significant increased incidence of hepatocellular carcinomas and adenomas in the high-dose group (1000 ppm) of female mice. There was no evidence of carcinogenicity at the other dose levels in females (50, 250 ppm) or in any dose group of male mice. Acephate was also tested in two-year feeding studies in Sprague-Dawley rats (Auletta *et al*., 1981; Knezevich and Hogan, 1982; both as cited by U.S. EPA, 1999). The incidence of benign adrenal pheochromocytoma was increased in all three groups of treated males. U.S. EPA considered the finding to be "dose-unrelated" (control: 2.7%; 5 ppm: 9.7%; 50 ppm: 15.5%; 700 ppm: 12.2%) and noted that the incidences in the treated groups were all within the historical control range (0.7% - 20.3%). There was no evidence of carcinogenicity in female rats. U.S. EPA (1999) reported that the authors did not provide justification for the dose selection in either of the two sets of cancer bioassays. Acephate is classified as a group C, possible human carcinogen (U.S. EPA, 1999; U.S. EPA, 2000).

U.S. EPA (1999) summarized data on the genotoxicity of acephate, based on 14 submitted studies characterized as "acceptable." The purity of acephate in most of these studies was 93-99%. *In vitro* studies indicated that acephate was genotoxic in bacteria, yeast, and cultured mammalian cells. Unscheduled DNA synthesis was induced by acephate in human fibroblasts. U.S. EPA (1999) stated that the *in vitro* genotoxicity was generally observed at "high concentrations" and did not require exogenous metabolic activation. Acephate was negative in a dominant lethal assay in CD-1 mice and a micronucleus assay in Swiss mice. U.S. EPA (1999) concluded that these negative *in vivo* findings "lessen the concern for a potential mutagenic hazard." The California Department of Pesticide Regulation (CDPR, 2002a) similarly concluded that there is evidence for the genotoxicity of acephate in *in vitro* studies, while i*n vivo* chromosome studies submitted by the registrant were negative. The *in vivo* tests included dominant lethal, micronucleus, bone marrow chromosomal aberration and SCE assays in mice and an assay for chromosomal aberration and sister chromatid exchange in phytohemagglutin-stimulated peripheral blood lymphocytes from monkeys (1/sex/group) exposed for 20 days. CDPR (2002a) noted that none of the *in vivo* studies "included good evidence that the bone marrow was exposed to a meaningful dose." Behera and Bhunya (1989) tested commercial grade acephate (75% pure) in a battery of *in vivo* tests in mice, including bone marrow chromosomal aberration, micronucleus, sperm-shape abnormality, and dominant lethal assays. The authors reported positive results in all the assays. More recently, the pesticide was reported positive for transformation of BALB/c 3T3 cells, clone A31 in the presence and absence of metabolic activation (Perocco *et al*., 1996). In a study examining alterations of biochemical markers related to "non-genetic cocarcinogenesis," acephate was found to modulate organ and sex-related differences in the induction and suppression of murine cytochrome P-450s (Paolini *et al*., 1997).

There is a **HIGH** level of **concern over the extent of exposure** to acephate. Acephate is a broad-spectrum organophosphate insecticide currently registered for use on a variety of fruit and vegetable crops; in food handling establishments; on ornamental plants and in and around the home. In California, approximately 240,000 pounds of acephate were used in agricultural and commercial applications in 2001 (CDPR, 2002b). Californians would be expected to be exposed to acephate via agricultural work and residues in food and water. According to a recent U.S. EPA exposure assessment, a high to medium concern may be expected for people consuming drinking water derived from surface water and for workers handling acephate on turf, floral crops and in residences (U.S. EPA, 2001).

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CARCINOGENICITY DATA SUMMARY: *TRANS*-ANETHOLE

Preliminary evaluation of carcinogenicity and exposure data

***trans*-Anethole** (*trans*-4-methoxy-1-propenylbenzene CAS No. 4180-23-8) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list.** No human carcinogenicity studies of *trans*-anethole were identified. Studies in both mice and rats were predominantly negative. This may in part be due to the limited studies for carcinogenicity and the weak hepatocarcinogenicity of this naturally occurring alkylbenzene derivative.

In a study of structure activity relationships on hepatocarcinogenicity for twenty-three alkylbenzene derivatives, Miller *et al*. (1983) found *trans*-anethole did not induce liver tumors in CD-1 mice after 12 months of daily administration, nor in male CD-1 or $B6C3F_1$ mice given the compound during the pre-weaning period. Truhaut *et al*. (1989) fed 0, 0.25, 0.5 and 1% *trans*-anethole to male and female CD rats for 117 weeks. In female rats there was an increased low incidence of benign and malignant hepatocellular neoplasms in the high-dose group. The histopathological findings for these tumors were re-evaluated by a pathology working group; the group concurred with the findings (Newberne *et al.,* 1989). Other hepatic lesions related to carcinogenesis were observed in the Truhaut study: altered cell foci in high dose females, nodular hyperplasia in mid dose males and high dose males and females. Lung tumors were not induced in A/He mice, a strain sensitive to lung tumor induction, administered multiple injections of *trans*-anethole for eight weeks and followed for 24 weeks (Stoner *et al*., 1973).

No genotoxicity data on *trans*-anethole exposure in humans either *in vivo* or *in vitro* were identified. A report of micronucleus assays in mice after intraperitoneal (i.p.) injection or oral administration was negative (Marzin, 1979; as cited by Truhaut *et al.*, 1989). Very low levels of DNA adduct formation were reported by Phillips *et al*. (1984) and Randerath *et al*. (1984) following i.p. administration to neonatal or adult mice. *Salmonella typhimurium* assays were generally negative in strains TA 98, TA 100, TA 1537, TA 1538 without metabolic activation or weakly mutagenic following activation (Marcus *et al*., 1982; Swanson *et al*., 1979; To *et al*., 1982; Gorelick, 1985). In one report, a positive response was observed in *Salmonella typhimurium*, however assays in *Bacillus subtilis* DNA-repair deficient strains and in *Escherichia coli uvr* reversion were negative (Sekizawa and Shibamoto, 1982). *trans*-Anethole did not induce chromosome aberrations in the Chinese hamster ovary assay (Gorelick, 1985). A dose-related response was reported in the L5178Y mouse lymphoma TK+/- assay with metabolic activation. The study author noted this was consistent, given other genotoxicity findings, with either a recombination event or a non-DNA reactive mechanism (Gorelick, 1985).

trans-Anethole belongs to a group of naturally occurring alkylbenzenes that also includes safrole, isosafrole, estragole, eugenol, methyleugenol, myristicin, elemicin, and dill and parsley apiols. Estragole, safrole, isosafrole, and methyleugenol are listed as carcinogens under Proposition 65. Estragole and methyleugenol have been found to induce hepatic tumors in carcinogenesis studies in which *trans*-anethole was inactive. The main structural difference between *trans*-anethole and its closest carcinogenic analog, estragole, is the location of the double bond in the hydrocarbon side chain. This structural difference may account for the observation that *trans*-anethole does not form DNA adducts as was reported by Phillips *et al.* (1984) and Randerath *et al.* (1984), and was also found to be only weakly mutagenic or carcinogenic. It is however noteworthy that *trans*-anethole epoxide, potentially a metabolite of *trans*-anethole, does induce hepatic tumors and skin papillomas in mice and is positive in *Salmonella* tester strains (Kim *et al.,* 1999).

trans-Anethole is "generally recognized as safe" by the U.S. Food and Drug Administration (FDA, 1994) and the Flavors and Extracts Manufacturers Association. *trans*-Anethole has not been evaluated by the International Agency for Research on Cancer or the U.S. Environmental Protection Agency.

There is a **HIGH** level of **concern over the extent of exposure** to *trans*-anethole. *trans*-Anethole is a natural flavor found in essential oils of plants including fennel, star anise and anise and is commonly used as a "natural" food additive in baked goods, sweets, and alcoholic and non-alcoholic beverages.

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CARCINOGENICITY DATA SUMMARY: ASPARTAME

Preliminary evaluation of carcinogenicity and exposure data

Aspartame [Equal®; NutraSweet®; L-aspartyl-L-phenylalanine methyl ester; CAS No. 22839-47-0] **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. There is, however, some carcinogenicity concern over observations of brain tumors in aspartame-treated rats. Reliable animal studies have not been conducted despite the widespread human exposure to this artificial sweetener. Epidemiologic data provide inadequate information on which to judge carcinogenicity. One small epidemiologic study found no evidence of an effect of aspartame consumption on brain tumor risk in children. Aspartame has been suggested as an explanation for increased rates of human brain cancer. Further epidemiologic and toxicologic studies are needed on the carcinogenicity of this chemical.

No large epidemiological studies of carcinogenicity have been conducted. Olney *et al*. (1996), performing a descriptive analysis of national cancer data, suggested the possibility that aspartame might be associated with increased incidence of brain tumors in the U.S. A small study (Gurney *et al*., 1997) of aspartame consumption in children and brain tumor risk found no evidence that cases (n=56) were more likely to consume foods containing aspartame than controls (n=90).

There have been multiple carcinogenicity studies of aspartame in animals, each of which is inadequate for judging carcinogenicity. Searle Laboratories has conducted two sets of studies in rats. In the first set, referred to as Study E-33/34, female and male Charles River CD Sprague-Dawley albino rats were fed 0, 1, 2, 4, or 8 g/kg aspartame daily for 104 weeks. In female rats, one 4 g/kg dose animal was observed with brain tumor (ependymoma) and three high dose females were (2 meningioma, 1 glioma) (Searle Laboratories, 1973). The brain tumor incidences in the Searle Laboratories (1973) report (number of tumors/number of animals examined) in the 0, 1, 2, 4, and 8 g/kg females were 0/59, 0/4, 0/4,1/4, 3/39, respectively, a statistically significant increase with increasing dose ($p = 0.0206$, Fisher Exact trend test; $p = 0.0167$, Cochran-Armitage trend test). In male rats, one brain tumor, a meningioma, was observed in the high dose group. The incidences were: 0/58, 0/4, 0/3, 0/1, 1/40 (Searle Laboratories, 1973). Searle Laboratories (1973) reported that these findings were not statistically significant (although Fisher Exact trend test for females indicates otherwise). The FDA Commissioner (1981) noted "variations in tumor count among the several persons or groups who viewed the slides." The FDA's Public Board of Inquiry (PBOI) reported the following brain tumors incidences (number of tumors/"total number of animals at risk"): females, 0/59, 2/40, 0/40, 1/40, 2/38; males, 1/59, 2/40, 0/40, 1/40, 2/38. These data, as reported by the PBOI, do not reflect the limited numbers of animals examined for brain histopathology in the low-, mid-, and midhigh-dose groups of both sexes, nor do these data reflect a significant increase in brain tumors with increasing dose in females. The PBOI expressed concern over the early occurrence of brain tumors in some animals (FDA Commissioner, 1981). There was disagreement among examining pathologists as to the positive finding in the male control group, with one of three finding no tumor (FDA Commissioner, 1981). The PBOI also considered historical background incidence of brain tumors in interpreting the study findings, and concluded that the available data did not rule out the possibility that aspartame might induce brain tumors (FDA Commissioner, 1981).

In the second set of Searle Laboratory studies, referred to as study E-70, aspartame was fed to female Charles River Sprague-Dawley rat dams during pregnancy and lactation and to their offspring after weaning for 104 weeks. Daily dose levels were 0, 2, and 4 g/kg. Five of 160 aspartame-fed rats and four of 120 controls were reported with brain tumors. Hyperplastic liver nodules were increased in treated females. An FDA review panel concluded that Searle Laboratories did not employ a feed analysis program to monitor their incorporation of test compound into feed. FDA's PBOI (Nauta *et al*., 1980) considered this a deficient study (FDA Commissioner, 1981).

Ishii (1981) fed groups of SCL Wistar rats 0, 1, 2 or 4 g/kg aspartame, or 4 gm/kg aspartame + diketopiperazine (DKP) (3:1) for 104 weeks and evaluated brain tumorigenicity. Interim sacrifice included 10 animals/sex/group at 26 weeks and 16 animals/sex/group at 52 weeks. No brain tumors were observed in the interim sacrifice animals. Total number of animals in the main groups was 60 sex/group; the number surviving to 104 weeks was reduced in some groups to as few as 16 (1 g/kg males), and in all groups was less than 30 in males and lower in males than females. Among females, one control had an "atypical astrocytoma"; two brain tumors were found at 2 g/kg (1 astrocytoma, 1 ependymoma) and one at 4 g/kg (oligodendroglioma). In males, one treated at 1 mg/kg was found with oligodendroglioma and one at 4 g/kg with astrocytoma.

Studies in mice fed aspartame in diet found no indication of increased tumor incidence (FDA Commissioner, 1981). Details of study results have not been published.

The National Toxicology Program (NTP, 2003a) has conducted non-standard bioassays in both sexes of genetically altered (p53 haploinsufficent) mice. Animals in groups of 15 were fed aspartame for nine months at feed concentrations ranging from 3,125 to 50,000 ppm. There was no evidence of treated-related carcinogenicity. This provides limited information on the potential for aspartame to induce cancer in humans; group sizes were small and the use of the genetically altered mouse is a new model. Thus, there is uncertainty as to whether the study possessed sufficient sensitivity to detect a carcinogenic effect (NTP, 2003b).

Aspartame breaks down spontaneously to diketopiperazine (DKP), which normally comprises less than 2% of the final aspartame product (FDA Commissioner, 1981). DKP was tested for brain tumorigenic activity in Sprague-Dawley rats fed DKP for 115 weeks (FDA Commissioner, 1981), in a study referred to as E-77/78, at doses of 0, 0.75, 1.5, and 3.0 g/kg. No increased incidence of brain tumors compared to untreated rats was observed. An FDA inspection team investigated the laboratory carrying out this study and found irregularities that included evidence of improper feed mixing (the chow was ground to a fine powder, but the DKP was present in large chunks), which may have allowed the rats to avoid eating the DKP (Bressler, 1977). The team also noted methodological quality control issues that could impact on the study findings.

The promoting potential of aspartame on urinary bladder carcinogenesis, initiated with N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), was studied in male F344 rats who received 0.01% BBN in drinking water for four weeks followed by 5% aspartame in the diet for 32 weeks (total aspartame intake, 400 gm/kg). The incidences of bladder lesions were not increased in the 28 rats surviving to the end of the experiment, 36 weeks (Hagiwara *et al*., 1984).

Aspartame was not mutagenic in TA 100 and TA 98 *Salmonella* tester strains (Shephard *et al*., 1993). Aspartame, nitrosated *in vitro* (to simulate the nitrosation that occurs in the stomach), was mutagenic towards TA100, TA104, and TA98 without metabolic activation, but not toward TA102 (Shephard *et al*., 1993). Aspartame was not clastogenic, *in vivo*, in mice (Durnev *et al*., 1995). Jeffrey and Williams (2000) reported that aspartame *in vitro* did not induce DNA synthesis in rat hepatocytes. Mukhopadhyay *et al.* (2000) report *in vivo* co-exposure of aspartame and acesulfame potassium was negative for the induction of chromosome aberrations in male Swiss mice bone marrow cells. Aspartame adducts were found in nucleic acids and proteins from aspartame-fed rats, and the authors concluded aspartame-derived formaldehyde was responsible for adduct formation (Trocho *et al*., 1998).

There is a **HIGH** level of **concern over the extent of exposure** to aspartame. Aspartame is a low-calorie sweetener, first approved in 1981, currently consumed by more than 100 million people around the world (Calorie Control Council, 2002). In the U.S., aspartame is available for use in more than 1500 products, including table-top sweeteners, carbonated beverages, baked goods, chewable multi-vitamins, hot and cold breakfast cereals, chewing gum, puddings and fillings, candies, cough drops, pharmaceuticals, and many other products (Calorie Control Council, 2002). The "acceptable daily intake" of aspartame, established by FDA, is 50 mg/kg; a food intake survey conducted by U.S. Department of Agriculture found some people in the U.S. consumed more than 16 mg/kg/day (Butchko *et al*., 1994).

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CARCINOGENICITY DATA SUMMARY: CHLOROACETIC ACID

Preliminary evaluation of carcinogenicity and exposure data

Chloroacetic acid [monochloroacetic acid; CAS No. 79-11-8] **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list.** There is no evidence of carcinogenicity from animal cancer bioassays, nor is there evidence from short-term tests of genotoxic activity at noncytotoxic concentrations. No human carcinogenicity data were identified.

No positive statistically significant bioassay results have been reported for chloroacetic acid, which has been tested in both rats and mice. Two-year oral gavage studies in male and female Fischer 344N rats (NTP, 1992) and drinking water studies in male Fischer 344N rats (DeAngelo *et al.,* 1997) have been conducted. Mouse studies include two-year oral gavage studies in $B6C3F_1$ mice of both sexes (NTP, 1992), 18-month oral gavage and combined diet studies in (C57BL/6 X C3H/Anf) F1 mice of both sexes (Innes *et al.,* 1969), and a 580-day skin painting study in female ICR/HA Swiss mice (Van Duuren *et al.,* 1974).

Chloroacetic acid was negative in a histidine reverse gene mutation assay in *Salmonella typhimurium* (one or more of the five standard strains: TA98, TA100, TA1535, TA1537, and TA1538) (GENE-TOX, 1995). Chloroacetic acid did not induce DNA strand breaks in the liver of rats following a single administration of 1-10 mmole/kg; it also failed to induce DNA strand breaks in splenocytes and epithelial cells derived from the stomach and duodenum of mice treated *in vivo* (Chang *et al*., 1992). Chloroacetic acid was ineffective in inducing DNA strand breaks in cultured rat and mouse hepatocytes at concentrations below those that yielded cytotoxicity (Chang *et al.,* 1992).

The U.S. Environmental Protection Agency (U.S. EPA, 2003) has not classified chloroacetic acid for potential carcinogenicity.

There is a **HIGH** level of **concern over the extent of exposure** to chloroacetic acid, which is found as a chlorine disinfection by-product in drinking water supplies (NTP, 1992). Chloroacetic acid used as an intermediate for production of various types of chemicals (HSDB, 2002). NTP (1992) and HSDB (2002) report that it is also used as a herbicide, however no active products were found in California (California Department of Pesticide Regulation, 2003).

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CARCINOGENICITY DATA SUMMARY: CHLOROMETHANE (METHYL CHLORIDE)

Preliminary evaluation of carcinogenicity and exposure data

Chloromethane (methyl chloride; CAS No. 74-87-3) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. There is, however, some concern based on findings of renal tumors in male mice, and observations of genotoxicity.

As reviewed by the International Agency for Research on Cancer (IARC, 1999), multiple small cohort studies of workers from facilities using or producing chloromethane showed no clear cancer excess. Holmes *et al*. (1986) did not find excess cancer mortality in a population of chloromethane-exposed male employees of a synthetic rubber manufacturing plant compared to the general U.S. population, although a slightly elevated rate of lung cancer, statistically nonsignificant, was seen among non-white employees, based on a small number of cases (n=6). In another cohort study of a chemical manufacturing facility, three pancreatic cancers were observed, where 0.9 was expected (Ott *et al.,* 1985). The workers were exposed to other chemicals and there was little information regarding the degree of chloromethane exposure. A third cohort study (Olsen *et al*., 1989, as reviewed by U.S. EPA, 2001) of chemical workers in Louisiana with exposures to chloromethane and multiple other chemicals observed a statistically nonsignificant increase in brain and other central nervous system cancer mortality, as compared with the U.S., Louisiana, or local referent populations (SMR: 333, 354, 322, respectively). Rafnsson and Gudmundsson (1997) reported on cancer incidence and mortality from long-term follow-up of a fishing vessel crew of 27 people accidentally exposed to chloromethane compared to a reference group of men with similar occupations, social class, and age; crude rate ratios for all cancers and lung cancer were elevated but not statistically significant, based on very small numbers of cases.

Two-year inhalation bioassays in mice and rats of both sexes were conducted with doses of 0, 50, 225 and 1000 ppm. A statistically significant increased incidence of malignant and benign (combined) renal tumors in male $B6C3F_1$ mice was seen at the highest concentration, 1000 ppm, and a non-significant increased incidence was seen at 225 ppm (U.S. EPA, 2001). No increase in treatment related tumors was observed in female B6C3F1 mice or in male or female Fischer 344 rats tested up to 1000 ppm (U.S. EPA, 2001).

The U.S. Environmental Protection Agency (U.S. EPA, 2001) characterized chloromethane as a possible human mutagen. In summarizing the genotoxicity studies, U.S. EPA (2001) noted the following findings. Chloromethane induced reverse mutations in *Salmonella typhimurium* in a dose-dependent manner; it also induced forward mutations in *Salmonella typhimurium*. In a human lymphoblast cell line, chloromethane induced mutations and sister chromatid exchange, but not alkaline-labile DNA single strand breaks. The *in vivo* induction of unscheduled DNA synthesis (UDS) has been observed in rat liver following exposure to relatively high, but not lower concentrations of chloromethane. Similarly, *in vitro* induction of UDS has been observed in rat spermatocytes and tracheal cells, and in primary cultures of human hepatocytes at relatively high, but not lower doses. Chloromethane induced sex linked recessive lethal mutations in *Drosophila melanogaster*, and was positive in the dominant lethal assay in Sprague-Dawley and F-344 rats. Chloromethane did not alkylate DNA *in vivo*, as administration of ^{14}C-labeled chloromethane by inhalation to mice and rats did not lead to methylation of DNA bases (Bolt and Gansewendt, 1993). Studies by Ristau *et al.* (1989, 1990) and Jäger *et al.* (1988) indicated that chloromethane induced DNA protein crosslinks and single strand breaks in male mouse kidney.

The U.S. EPA (2001) concluded that the potential for human carcinogenicity of chloromethane cannot be determined. IARC (1999) classified chloromethane as Group 3; not classifiable due to inadequate evidence for carcinogenicity in humans and in experimental animals.

There is a **HIGH** level of **concern over the extent of exposure** to chloromethane. It is produced and used primarily in the manufacture of silicone rubber; previously chloromethane was used as a refrigerant and anesthetic (U.S. EPA, 2001). The National Institute for Occupational Safety and Health (NIOSH, 1984) estimated that in 1983 approximately 10,000 U.S. workers were exposed to chloromethane. Projected demand for chloromethane in 2001 was 775 million pounds (HSDB, 2003). In addition to occupational exposures to chloromethane, the compound is the most common halogenated hydrocarbon found in the atmosphere and is found in chlorinated drinking water. The oceans are the largest source of atmospheric chloromethane.

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CARCINOGENICITY DATA SUMMARY: CHOLESTYRAMINE

Preliminary evaluation of carcinogenicity and exposure data

Cholestyramine (CAS No. 11041-12-6) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, although there is some concern.

The Lipid Research Clinics (LRC) Coronary Primary Prevention Trial (CPPT) was designed to test whether the lowering of plasma levels of low-density lipoprotein (LDL) via administration of cholestyramine would reduce the incidence of coronary heart disease events (LRC Investigators, 1992). 1907 men received cholestyramine for 7.4 years, while 1899 men received a placebo. At the end of the trial, cancers of the mouth and pharynx, pancreas and urinary bladder, and colorectal tumors were observed more frequently in the cholestyramine group, with melanoma and lung cancer observed more frequently in the placebo group. After six years of post-trial follow-up, the incidences of benign colorectal tumors and cancer of the buccal cavity and pharynx were nonsignificantly increased in the cholestyramine group. The risk of colorectal cancer was not related to cumulative dosage ("packet-years") of cholestyramine. The authors considered it biologically plausible that resin exposure could be associated with colorectal tumors. However the authors also noted that there might have been ascertainment bias, in that gastrointestinal symptoms in patients taking the resins might have resulted in diagnostic procedures being conducted that incidentally detected the colorectal polyps which would not be detected in patients not taking resins. In terms of the buccal cavity and pharynx cancers, the authors postulated a potential cocarcinogenic effect of cholestyramine with cigarette smoke. The authors noted however that the incidence of these cancers in the cholestyramine group "did not exceed the expected incidence in comparably aged US men followed up over the same time period and the incidence of other more lethal tobacco-related cancers (*e.g.*, lung cancer) was lower in the cholestyramine than in the placebo group." The length of follow-up may not have been sufficient to detect carcinogenic effects; additional follow-up of this study is planned.

Takeuchi *et al*. (1982; paper in Japanese, English abstract reviewed) exposed male and female B6C3F_1 mice to 1.25, 2.5 and 5% cholestyramine in the diet for 18 months, followed by three months of normal diet. Mortality was significantly increased in the high dose group of male mice. Tumor incidence in the low and mid-dose groups was similar to controls. Tumor incidence in mice exposed to 5% cholestyramine was lower than the other three groups.

Takeuchi *et al.* (1983; paper in Japanese, English abstract reviewed) exposed male and female Fischer rats to 1.25, 2.5 and 5% cholestyramine in the diet for 26 months, followed by two months of normal diet. High dose male rats exhibited a lower mean survival time compared to controls. No treatment-related increase in tumors was observed.

Numerous initiation-promotion studies of cholestyramine have been conducted, with mixed results (Asano *et al.*, 1975; de Heer *et al.*, 1981; Ikematsu *et al*., 2000; Kishinaka *et al*., 1998; Melhem *et al*., 1987; Morgan *et al*., 1990; Nigro *et al*., 1973; Ogawa *et al.*, 1992). In most cases cholestyramine promoted carcinogenesis, but some studies showed an inhibitory effect while others showed no effect. No initiation-promotion studies were identified that showed a carcinogenic effect associated with exposure to cholestyramine alone.

There is a **HIGH** level of **concern over the extent of exposure** to cholestyramine. It is a cholesterol-lowering drug approved for use in the U.S. (U.S. Food and Drug Administration, 2003).

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CARCINOGENICITY DATA SUMMARY: CLOFENTEZINE

Preliminary evaluation of carcinogenicity and exposure data

Clofentezine (Apollo™; 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine; CAS No. 74115-24-5) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, but there is some carcinogenicity concern. Clofentezine induced a significant increase in combined benign and malignant thyroid gland tumors in male rats treated in the high-dose group of a three-dose study conducted for up to 27 months, with a significant positive trend (FBC/Nor-Am Chemical Co, 1985; as described by the U.S. Environmental Protection Agency [U.S. EPA], 1993). According to U.S. EPA (1999), the maximally tolerated dose may not have been achieved. A significant increase in the incidence of malignant and combined benign and malignant liver tumors was observed in the mid-dose group of male mice in a three-dose study (FBC/Nor-Am Chemical Co., 1985b; as described in U.S. EPA, 1993). Tempering the level of concern are the relatively small increases in incidences of thyroid and liver tumors and the absence of a clearly increasing dose-response relationship for liver tumors. Corresponding studies in female rats and mice were also reported to be negative, although high mortality occurred in the study of female mice. U.S. EPA has classified clofentezine (Apollo) in Group C, possible human carcinogen, based upon an increase in thyroid gland follicular cell tumors in male rats and supportive findings in pituitary/thyroid hormone activity (U.S. EPA, 1993). U.S. EPA has also published as a final rule, a pesticide tolerance for clofentezine in which the compound is classified as "a likely human carcinogen (classification of C)" (U.S. EPA, 1999).

Clofentezine has tested negative in *Salmonella* reverse mutation assays (with and without hepatic homogenate), negative in gene conversion / mitotic recombination assays in *Saccharomyces cerevisiae* strain D7, and negative for dominant lethal mutations in male rats following 10 weeks of treatment at 400 ppm. A mouse lymphoma assay was reported to be equivocal. U.S. EPA (1993) also noted a lack of structurally similar carcinogens.

Several observations of the effects of clofentezine have suggested that a thyroid-hormone depletion mechanism by the liver may be responsible for the effects on the thyroid. These effects include stimulation of mixed-function oxidases, uridine diphosphate glucuronosyltransferase, and increases in biliary flow and excretion (Hurley, 1998).

There is a **HIGH** level of **concern over the extent of exposure** to clofentezine. Clofentezine is a pesticide primarily used for the control of mites on nut and fruit trees. Clofentezine is the active ingredient in pesticide products that are currently registered in California (Apollo SC: 42% clofentezine). The California Pesticide Use Report for the year 2001 indicates that approximately 3900 pounds of clofentezine were applied in over 1700 applications (CDPR, 2002). A 1992 report from the Food and Agriculture Organization of the United Nations reported clofentezine residues in a number of crops treated with the pesticide (FAO, 1992). The international studies reviewed in this report found clofentezine residues ranging from 0.03 to 1.2 ppm on grapes, 0.1 to 0.43 on oranges and lemons nearly a month following treatment.

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CARCINOGENICITY DATA SUMMARY: CYCLOATE

Preliminary evaluation of carcinogenicity and exposure data

Cycloate [s-ethyl cyclohexylethylthiocarbamate; CAS No 1134-23-2] **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, although positive *in vitro* genotoxicity tests and preneoplastic lesions induced in chronic studies are cause for concern.

In dietary carcinogenicity studies in male and female rats (Kundzins, 1979; Sprague, 1984) and in male and female mice (Goldenthal, 1979; Stonard, 1991), there was no evidence of compound-related oncogenic effects (as reviewed by the California Department of Pesticide Regulation [CDPR], 1998). However, in a rat subchronic inhalation study, several potential preneoplastic changes in the nasal cavity (goblet cell hyperplasia, respiratory epithelial cell hyperplasia, and transitional cell hyperplasia), and in the larynx (squamous epithelial cell hyperplasia) were observed (as reviewed by CDPR, 1998).

The U.S. Environmental Protection Agency (U.S. EPA) does not treat cycloate as a carcinogen. In 1994, U.S. EPA added the chemical to its Toxic Release Inventory list of chemicals based on developmental and neurotoxicity, not cancer concerns (U.S. EPA, 1994).

Some concern is supported by *in vitro* studies that show that cycloate has the potential to cause cytogenic damage. Increases in the frequencies of mutation, sister chromatid exchange and chromosomal aberrations were observed in mouse lymphoma (L5178Y) cells in the presence of metabolic activation. However, studies involving gene mutation in *Salmonella typhimurium* and *Saccharomyces*, as well as chromosomal aberration studies using the mouse micronucleus assay and human lymphocyte cultures, were negative (as reviewed by CDPR, 1998).

There is a **HIGH** level of **concern over the extent of exposure** to cycloate. Cycloate, a thiocarbamate pesticide, is a selective herbicide for pre-plant use to control many broad leaf weeds, annual grasses, and nutsedge in fields for sugarbeets, table beets, and spinach. CDPR (2002) reported that approximately 32,000 pounds were used in California in 2001.

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CARCINOGENICITY DATA SUMMARY: 3,4-DIHYDROCOUMARIN

Preliminary evaluation of carcinogenicity and exposure data

3,4-Dihydrocoumarin [CAS No. 119-84-6] **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, although there is some carcinogenicity concern. The chemical induced benign liver tumors in female mice and benign renal tumors in male rats.

Animal bioassays were carried out by the National Toxicology Program (NTP 1993a) in B6C3F_1 mice and Fischer 344 rats of both sexes. Hepatocellular adenomas were observed to be increased in treated female mice and renal adenomas in treated male rats. Focal hyperplasia of the kidney was also observed in male rats. A long-term study by Dickens and Waynforth (1968) of subcutaneous injection of 3,4-dihydrocoumarin in small numbers of rats and mice produced no increases in tumors (NTP, 1993a).

No human cancer epidemiology data are available for 3,4-dihydrocoumarin.

Chinese hamster ovary cells exhibited increased sister chromatid exchange, but not chromosomal aberrations, upon exposure to 3,4-dihydrocoumarin with or without metabolic activation (NTP, 1993a). Mouse micronucleus assays were negative after 13 weeks of exposure (NTP, 1993a). Gene mutations were not observed in TA98, TA100, TA1535, or TA1537 strains of *Salmonella typhimurium* exposed to 3,4-dihydrocoumarin with or without metabolic activation (NTP, 1993a; Prival *et al.*, 1982; Haworth *et al.*, 1983). As noted by NTP (1993a), the genetic toxicity data indicate little potential for direct interaction with cellular DNA.

3,4-Dihydrocoumarin is structurally related to coumarin, a mutagenic chemical that caused lung cancer in female mice (NTP, 1993b). It has been hypothesized that the different toxicity is because coumarin is metabolized to a 3,4-epoxide intermediate but 3,4-dihydrocoumarin, which lacks a 3,4-double bond, is not (Born *et al*., 1998, Gu *et al*., 1997).

There is a **HIGH** level of **concern over the extent of exposure** to 3,4-dihydrocoumarin. It is used as a flavoring agent in sweets and a fragrant in beauty and skin care products. Occupational exposure is thought to be minimal. The combined domestic production and imports have been estimated at 50 tons per year (HSDB, 2002). The National Institute for Occupational Safety and Health found that between 1981 and 1983, only 2,054 workers were potentially exposed to 3,4-dihydrocoumarin (NTP, 1993a).

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CARCINOGENICITY DATA SUMMARY: FLUTAMIDE

Preliminary evaluation of carcinogenicity and exposure data

Flutamide (2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide; CAS No. 13311-84-7) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list,** however, there is some concern from studies in animals. Flutamide is an anti-androgen that is used in conjunction with luteinizing hormone-releasing hormone (LHRH) agonists [such as Leuprolide] for treatment of metastatic prostate carcinoma. Flutamide induces Leydig cell tumors in rats (Prentice and Meikle, 1995; PDR, 2002), presumably due to sustained hypersecretion of luteinizing hormone (Cook *et al*., 1993).

The potential carcinogenicity of flutamide has not been adequately studied epidemiologically. Cases of malignant male breast cancer have been reported in patients following treatment with flutamide for prostate cancer (Emoto *et al*., 2001; PDR, 2002). Gynecomastia is a side effect of flutamide usage (Staiman and Lowe, 1997). This may have some relevance to the case reports. Flutamide has also been used in the treatment of male breast cancer (Labrie *et al*., 1990). The cancer registry for flutamide has not shown increased incidence of Leydig cell adenomas (of the testes), but the possibility of Leydig cell hyperplasia and adenomas cannot be ruled out currently (Clegg *et al*., 1997, Cook *et al*., 1999).

Daily administration of flutamide to male rats for one year at doses one to four times human therapeutic dose rates produced testicular interstitial cell adenomas in all treated groups (incidences ranging between 49 and 75%) (PDR, 2002). In male rats exposed to similar doses for one year followed by a one year period of no exposure, testicular interstitial cell adenomas were still present, although at somewhat lower incidences (43 to 47%). In a 24-month carcinogenicity study in male rats conducted using the same doses, high incidences of testicular interstitial cell adenomas were observed in treated male rats (91 to 95% compared to 11% in controls). Mammary gland adenoma, adenocarcinoma, and fibroadenoma were also increased in male rats (PDR, 2002).

In vitro studies of human hepatocytes exposed to flutamide concentrations ranging from 18 to 56 μM did not induce DNA fragmentation or DNA repair synthesis (Martelli *et al*., 2000). In rats administered a single oral dose of 500 mg/kg flutamide, there was no observed fragmentation or repair of DNA and there was no increase in the frequency of micronucleated hepatocytes (Martelli *et al*., 2000). Tests for induction of dominant lethal mutations in rats were negative and flutamide was not mutagenic in *Salmonella typhimurium* (PDR, 2002).

There is a **HIGH** level of **concern over the extent of exposure** to flutamide. Flutamide is an anti-androgen used in combination with Leuprolide to suppress testosterone production in patients being treated for metastatic prostate carcinoma.

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CARCINOGENICITY DATA SUMMARY: ISONIAZID

Preliminary evaluation of carcinogenicity and exposure data

Isoniazid (CAS No. 54-85-3) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. There are a number of positive studies in mice and one positive study in rats. Epidemiological data have been generally negative.

One epidemiology study reported a slight excess of deaths from malignant neoplasms of the bronchus, lung and pleura in 2696 patients treated with isoniazid compared with 1146 patients not treated with isoniazid that had no excess of these cancers (Stott *et al*., 1976). However, no dose-response effect was seen for either the total consumption or maximum daily dose of isoniazid. Four other cohort studies failed to find a statistically significant increase in lung cancer or cancer as a whole among isoniazid treated patients (Glassroth *et al*., 1977a; Howe *et al*., 1979; Boice and Fraumeni, 1980; Costello and Snider, 1980). In addition, five case-control studies provided no evidence of risk associated with isoniazid therapy (Zheng *et al*., 1987; Glassroth *et al*., 1977b; Miller *et al*., 1978; Kantor *et al*., 1985; Sanders and Draper, 1979). IARC (1973, 1987a) concluded that isoniazid is not classifiable as to its carcinogenicity in humans (Group 3). Stolley and Zahm (1995) note that although isoniazid is carcinogenic in animals, epidemiologic studies have shown little or no evidence of carcinogenicity. Mesothelioma following pre-natal exposure to isoniazid has been reported (Tuman *et al*., 1980; Fraire *et al*., 1988), but was not thought to be causally related to isoniazid exposure (Fraire *et al*., 1988).

In mice, lung tumors are induced after oral, intraperitoneal, or subcutaneous administration of isoniazid. Eighteen studies conclude that isoniazid is carcinogenic in mice (reviewed in IARC, 1973 and 1987a). In addition, the major metabolite of isoniazid, 1-acetyl-2-isonicotinoylhydrazine is carcinogenic in mice (Toth and Shimizu, 1973). However, studies in rats and hamsters were generally negative. Five studies in rats were negative (Loscalzo, 1964 and Lucchesi *et al*., 1967, as reviewed by IARC, 1973; Gershbein and Rao, 1992; Peacock and Peacock, 1966; Toth and Toth, 1970) while one set of long-term oral studies in rats demonstrated lung and liver tumors in males and mammary fibroadenomas in females (Severi and Biancifiori, 1968). A possible explanation for the high incidence of tumors in mice relative to other rodents is based on pharmacokinetics of isoniazid metabolism. It has been hypothesized that mice are slow acetylators of isoniazid and that the drug persists in mouse circulation for an extended period of time (Bhide *et al*., 1981).

In a review of genotoxicity data for isoniazid by IARC (1987b) no chromosomal aberrations (CA) were detected in peripheral blood lymphocytes from treated patients. Tests of CA and sister chromatid exchanges (SCE) in cultured lymphocytes treated with isoniazid were inconclusive but no unscheduled DNA synthesis was induced. No genotoxicity was induced in rodents *in vivo* while CA and SCE were induced in cultured rodent lymphocytes. No transformation of Syrian hamster embryo cells was observed and there was no gene conversion in yeast. Isoniazid was mutagenic in *Salmonella typhimurium* repair deficient strains TA100 and TA 1535 in the absence, but not the presence of rat S-9 liver microsomal fraction (Braun *et al*., 1984). Isoniazid was not mutagenic in frameshift mutation sensitive *Salmonella typhimurium* strain TA1538 (Malca-Mor and Stark, 1982). Isoniazid was not mutagenic in *Escherichia coli* (IARC, 1987b).

There is a **HIGH** level of **concern over the extent of exposure** to isoniazid. Isonaizid is used as an antibacterial agent primarily for the treatment of tuberculosis in humans. In addition, isoniazid is often administered prophylactically to exposed individuals, particularly those infected with HIV.

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CARCINOGENICITY DATA SUMMARY: LEVOBUNOLOL AND ITS SALTS

Preliminary evaluation of carcinogenicity and exposure data

Levobunolol [Betagan®, (-)-5-[3,(t-butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2H)-naphthalenone, CAS No. 47141-42-4] **and its salts did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. There is some concern, however, based on observations in long-term studies in mice and rats (Rothwell et al., 1992) of uterine leiomyomas (a benign tumor) in female mice and benign liver tumors in male rats.

Male and female CF1 mice (50/sex for levobunolol-treated groups and 100/sex for controls) were administered levobunolol via the diet at doses equivalent to 0, 12, 50, or 200 mg/kg body weight. The incidence of uterine leiomyomas among high dose female mice was 8.0 percent compared to zero in controls ($p<0.05$). Male and female Wistar rats (70/sex for levobunolol-treated groups and 110/sex for controls) were administered levobunolol via the diet for two years at doses of 0, 0.5, 2, or 5 mg/kg body weight. Incidences of benign hepatoma were 1.7, 2.9, 2.9, or 10 percent, respectively. Incidence in the highest dose group was significantly higher than that of controls ($p<0.05$). No increases in tumors were observed in male mice or in female rats (Rothwell *et al.*, 1992).

According to a U.S. Food and Drug Administration approved product label for levobunolol (U.S. FDA, 2000), "Levobunolol did not show evidence of mutagenic activity in a battery of microbiological and mammalian *in vitro* and *in vivo* assays."

There is a **HIGH** level of **concern over the extent of exposure** to levobunolol and its salts. Levobunolol is a prescription drug administered as eye drops for the treatment of glaucoma (U.S. FDA, 2000) and has undergone clinical trials for treatment of hypertension, angina pectoris, and arrhythmia (Rothwell *et al.*, 1992).

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CARCINOGENICITY DATA SUMMARY: METHYL METHACRYLATE

Preliminary evaluation of carcinogenicity and exposure data

Methyl methacrylate [methacrylic acid, methyl ester, CAS No. 80-62-6] **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. There is nonetheless some carcinogenicity concern based on findings of increased cancer incidence in some epidemiology studies.

Significantly increased rates of colorectal cancers among a cohort of methyl methacrylate-exposed workers has been reported (Walker *et al.*, 1991, as reviewed by IARC, 1994). In addition, in another cohort, increased findings of cancer of the female genital system and male lung (Fedotova *et al.,* 2000) and male colon (Fedotova *et al*., 2002) were reported. Statistically significant increases in tumor incidences were not observed in other worker cohorts exposed to methyl methacrylate (Collins *et al.*, 1989; Walker *et al.*, 1991, as reviewed in IARC, 1994 and U.S. EPA, 1998; Tomenson *et al.,* 2000). The International Agency for Research on Cancer (IARC, 1994) and the U.S. Environmental Protection Agency (U.S. EPA, 1998) both concluded that the human evidence is inadequate. The most recent epidemiological findings were not considered in reaching these conclusions.

Inhalation studies in male or female rats, mice, or hamsters showed no evidence of carcinogenicity treated via inhalation (Hazelton Laboratories, 1979 as reported by U.S. EPA, 1998; NTP, 1986). Also, tumors were not observed in rats treated with methyl methacrylate orally (Borzelleca *et al.*, 1964, as reviewed by IARC, 1994), topically (Oppenheimer *et al.*, 1955, as reported by IARC, 1979), or via subcutaneous implantation (Laskin *et al.*, 1954). Some of these bioassays were limited in power to detect effect due to small sample size and study duration.

IARC (1994) classified methyl methacrylate as Group 3 (not classifiable as to its carcinogenicity to humans). U.S. EPA (1998) classified methyl methacrylate as Group E (evidence of non-carcinogenicity to humans under its 1986 cancer guidelines) or "not likely to be carcinogenic to humans."

Methyl methacrylate induced clastogenic effects in mammalian cells *in vitro* (NTP, 1986; U.S. EPA, 1998). In *in vivo* studies, methyl methacrylate exposures did not cause clastogenic effects or dominant lethal mutations following inhalation or oral exposures to rodents (reviewed in IARC, 1994; U.S. EPA, 1998). Results from studies of chromosomal damage in exposed humans are equivocal (reviewed in U.S. EPA, 1998).

There is a **HIGH** level of **concern over the extent of exposure** to methyl methacrylate. Methyl methacrylate is widely produced for manufacturing acrylic sheeting and other polymers used in a variety of products including orthopedic surgery, dental prosthesis and synthetic fingernails. It is also used in polymer concrete, paints, and coatings (IARC, 1994).

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CARCINOGENICITY DATA SUMMARY: SYNTHETIC VITREOUS FIBERS:

ROCKWOOL (STONEWOOL)

SLAGWOOL

CONTINUOUS GLASS FILAMENTS

Preliminary evaluation of carcinogenicity and exposure data

In referring to this group of substances, OEHHA originally used the term "man-made mineral fibers"; these non-crystalline, fibrous substances made from rock, slag, glass or other processed minerals will now be referred to as "synthetic vitreous fibers." Other names for this group of substances include man-made vitreous fibers, manufactured vitreous fibers, and insulation wools. The five major fiber types included within this group of substances are glasswool (fiberglass), ceramic fibers (refractory ceramic fibers), rockwool (stonewool), slagwool, and continuous glass filaments (IARC, 2002). Other fiber types include newer and less well-studied synthetic vitreous fibers, such as alkaline earth silicate wools and high-alumina, low-silica wool fibers (IARC, 2002).

Two of the major fiber types, "glasswool (airborne particles of respirable size)" and "ceramic fibers (airborne particles of respirable size)," are already on the Proposition 65 list as chemicals known to the State to cause cancer. Of the three remaining major synthetic vitreous fiber types, **rockwool (stonewool), slagwool,** and **continuous glass filaments did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. There is nonetheless some carcinogenicity concern for rockwool and slagwool, based on multiple findings of genotoxicity *in vitro*, and tumor induction in experimental animals following intraperitoneal injection.

Recent updates of large retrospective cohort studies of workers exposed to synthetic vitreous fibers during the production of insulation products found no significant associations of cancer and fiber exposures in the U.S. (Marsh *et al*., 2001a,b,c; Stone *et al*., 2001) or in Europe (Boffetta *et al*., 1999; Plato *et al*., 1995). No significant increases in tumors were observed in rodent inhalation studies of rockwool or slagwool (reviewed in IARC, 1988; Ellouk and Jaurand, 1994; NRC, 2000; Kamstrup *et al*., 2001; NTP, 2002; IARC, 2002), although there are methodological concerns regarding many of the rodent inhalation cancer studies. Intraperitoneal injection of rockwool or slagwool to rats increased the incidence of mesothelioma in several studies (IARC, 2002). No rodent inhalation cancer studies of continuous glass filaments were identified (IARC, 2002). Intraperitoneal injection studies in rats of continuous glass filaments did not result in the induction of tumors (IARC, 2002). Rockwool and slagwool have tested positive in a variety of short-term tests for genotoxicity, including mutations in *Salmonella*, micronuclei and ploidy in CHO cells, and chromosomal aberrations, DNA breaks, DNA interstrand cross-links, and inhibition of DNA repair in cultured human cells (IARC, 2002). No studies of the genetic effects of continuous glass filaments were identified (IARC, 2002).

The carcinogenicity of synthetic vitreous fibers appears to depend primarily on (1) fiber dimensions and respirability and (2) biopersistence (Ellouk and Jaurand, 1994; NRC, 2000; Oberdörster *et al*., 2000; Moolgavkar, 2001). Differences in the chemical compositions of the synthetic vitreous fibers do not appear to play a significant role, except as the fiber chemistry pertains to the two factors above (Moolgavkar *et al*., 2001; NRC, 2000). Moolgavkar *et al.* (2001) analyzed experimental data from long-term bioassays in Fischer rats, and concluded that "the data are consistent with the hypothesis that all man-made mineral fibers have essentially the same oncogenic potential" and that "the oncogenic potential of man-made mineral fibers is determined mainly by their biopersistence." Studies that were considered negative were reinterpreted by Moolgavkar *et al.* (2001), and these authors concluded that the studies lacked the power to detect the level of risk associated with the level of exposure in those studies.

The National Toxicology Program (NTP) listed two of the major types of synthetic vitreous fibers, namely "Glasswool (respirable size)" and "Ceramic Fibers (respirable size)," as "*reasonably anticipated to be a human carcinogen*" in the NTP Report on Carcinogens (NTP, 2002). The International Agency for Research on Cancer (IARC, 2002) classified refractory ceramic fibers and special purpose glass fibers such as E-glass and '475' glass fibers as *possibly carcinogenic to humans (Group 2B)*, based on sufficient evidence in experimental animals. Insulation glasswool, rockwool, slagwool and continuous glass filaments were deemed *not classifiable as to their carcinogenicity to humans (Group 3)*, based on limited, or in the case of continuous glass filaments, inadequate evidence in experimental animals and inadequate evidence in humans (IARC, 2002).

There is a **HIGH** level of **concern over the extent of exposure** to rockwool (stonewool), slagwool, and continuous glass filaments. Synthetic vitreous (glass-like) fiber thermal and acoustical insulation products, including rockwool

(stonewool), slagwool, and continuous glass filaments, are used in buildings, vehicles, and appliances, and for high-temperature applications (*e.g.*, furnaces) (IARC, 2002). Other uses include filtration media, batteries, textiles, and reinforcement of plastics (IARC, 2002). Worker exposures are widespread (IARC, 1988); indoor and outdoor air concentrations are much lower in non-occupational settings, as compared to occupation settings (IARC, 2002).

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CARCINOGENICITY DATA SUMMARY: NICOTINE

Preliminary evaluation of carcinogenicity and exposure data

Nicotine (CAS No. 54-11-5) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list,** but there is some concern for carcinogenicity. Results from short-term studies suggest nicotine and its metabolites may affect gene expression, DNA synthesis, cell proliferation, and DNA fragmentation, and thus may play a role in carcinogenesis. Also, carcinogenic nitrosamines can be formed from nicotine during tobacco manufacture and use. The limited animal bioassay and genotoxicity data available do not provide evidence indicating nicotine is a carcinogenic or genotoxic hazard.

No tumors were observed in the cheek pouch or forestomach of male hamsters that were administered nicotine on the cheek pouches for 13 months (Chen and Squier, 1990; Chen *et al.*, 1994). Subcutaneous injections of nicotine were administered to pregnant rats (14-20 days post coitum) and their pups (1-20 days postpartum, 4-26 weeks) and observations continued until natural death (Berger *et al.*, 1987). No tumors were detected. No tumors were detected among male hamsters that received subcutaneous injections of nicotine for their lifetime (Schuller *et al.*, 1995). No lung tumors were observed among female rats exposed to nicotine by inhalation for two years, and incidences of tumors of the mammary gland, pituitary gland, ovary or skin were not significantly increased compared to control rats (Waldum *et al.*, 1996). No tumors were observed in a rabbit, in female guinea pigs or in male or female rats who received nicotine by subcutaneous injections (PHS, 1951; PHS, 1969). Boyland (1968) reported a study published in 1935 in which some rats exposed to nicotine by daily subcutaneous injections for 20 months developed "adenomata of the adrenal medulla."

Increased tumor incidences were observed in hamsters when nicotine was administered under hyperoxic conditions (Schuller *et al.*, 1995) or with 7,12-dimethylbenzanthracene (Chen and Squier, 1990). Decreased tumor incidences were observed among rats when nicotine was administered with methylnitrosourea (Berger *et al.*, 1987) and among mice when nicotine was administered after benzo[a]pyrene (PHS, 1971).

Nicotine increased DNA synthesis *in vitro* (Kozlovskis-Wade *et al.*, 1998) and increased proliferation in human ectocervical and papilloma virus transformed ectocervical cell lines but not in human endocervical or malignant cervical cells (Waggoner and Wang, 1994). DNA fragmentation was induced, *in vitro*, in nicotine treated human myelogenous leukemic cells, but not in human peripheral blood lymphocytes or polymorphonuclear cells (Yoshida *et al.*, 1998). Chowdhury *et al*. (1998) reported nicotine enhanced the expression of a mutant ras p21 protein and activated the H-ras gene in pancreatic acinar cells. Nicotine-induced sister chromatid exchange data were considered equivocal because of inadequate testing (Tucker *et al.*, 1993). Nicotine was not mutagenic towards various strains of *Salmonella typhimurium* in the absence or presence of metabolic activation (NTP, 1999; CCRIS, 1998) and did not induce aneuploidy or chromosomal aberrations in *Neurospora crassa* (GENE-TOX, 1998). Nicotine was not mutagenic in a bacterial luminescence assay whereas a metabolite, cotinine, elicited a positive response (Yim and Hee, 1995).

Major urinary metabolites of nicotine are cotinine and its hydroxylated derivative (Gorrod *et al.*, 1974; Allena *et al.*, 1999). Carcinogenic nitrosamines from nicotine may be formed in the presence of nitrate/nitrite during the manufacture and use of tobacco (Hoffmann and Hecht, 1985). Gorrod *et al*. (1974) reported that an increase in the ratio of the N-oxide of cotinine to that of nicotine may be associated with bladder cancer in humans.

There is a **HIGH** level of **exposure concern** for nicotine. Nicotine is a natural tobacco plant alkaloid and is found in cigarettes, cigars, pipe tobacco, snuff and other smokeless tobaccos (Hoffmann and Hecht, 1985; Hayes and Laws, 1991; Kyerematen and Vesell, 1991; Hoffmann *et al*., 1995; Jacob *et al*., 1999). Nicotine is used as a drug to aid smoking cessation and is available in patches, nasal sprays and lozenges (Foulds *et al.*, 1998, PDR, 1998a, PDR, 1998b). Nicotine has been measured in offices and smoking seats of trains and airplanes, in breast milk from smokers and nonsmokers and in serum of newborn infants nursed by smoking mothers. Children are exposed to nicotine and its metabolite cotinine via environmental tobacco smoke, as indicated by elevated levels of these chemicals in their urine (HSDB, 2003).

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CARCINOGENICITY DATA SUMMARY: 3-NITROFLUORANTHENE

Preliminary evaluation of carcinogenicity and exposure data

3-Nitrofluoranthene (CAS No. 892-21-7) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list.** There is nonetheless some carcinogenicity concern based on findings of injection site sarcomas in male rats following short-term exposure via subcutaneous injection, lung adenomas in newborn female mice following short-term exposure via intraperitoneal injection, genotoxicity in bacterial and mammalian cells, and binding with DNA, and structural similarities to known carcinogens.

Sarcomas, mainly malignant fibrous histiocytomas, were induced at the site of injection in male F344 rats who received 3-nitrofluoranthene by subcutaneous injection for 7.5 weeks and were observed for one year ($p<0.02$; 4/10 compared to 0/20 among controls) (Ohgaki *et al.*, 1982). Busby *et al*. (1989) reported an increased incidence and multiplicity of lung tumors among male and female newborn Swiss-Webster mice who received two doses of 3-nitrofluoranthene by intraperitoneal injection for two weeks and were observed for 26 weeks. Adenocarcinomas were observed in one low dose male, two high dose females, and one control male, and were thus not significantly elevated in treated animals. The incidences of lung tumors (adenoma or adenocarcinoma) in control, low and high dose animals were, respectively, 13/91, 7/29 and 7/25 in males and 7/101, 8/24 ($p=0.002$), and 9/27 ($p=0.001$) in females. A non-significant increase in squamous cell carcinoma and a marginal increase in Leydig cell tumors occurred in male F344 rats who received one pulmonary implantation of 3-nitrofluoranthene and were observed for 100 weeks (Horikawa *et al.*, 1991). A marginal increase ($p = 0.07$) was observed in the incidence of liver tumors in neonatal male $B6C3F_1$ mice administered 3-nitrofluoranthene by intraperitoneal injection at one, eight and 15 days following birth and then evaluated at one year (von Tungeln *et al.*, 1999). The authors reported no lung tumors were found in the 3-nitrofluoranthene treated mice.

3-Nitrofluoranthene did not induce micronuclei *in vivo* in mice (Tokiwa *et al.*, 1993). It was genotoxic, *in vitro*, towards Chinese hamster V79 cells (Berry *et al.*, 1985), human B-lymphoblastoid cells (Durant *et al.*, 1996), and Syrian hamster embryo cells (DiPaolo *et al.*, 1983). 3-Nitrofluoranthene induced unscheduled DNA synthesis in rat and mouse hepatocytes (Mori *et al.*, 1987) but not in human hepatocytes (Yoshimi *et al.*, 1987). Among the 3-nitrofluoranthene induced liver tumors, 4/9 exhibited mutations in *ras* protooncogenes; control data were not presented (von Tungeln *et al.*, 1999). 3-Nitrofluoranthene was mutagenic towards *Salmonella typhimurium* TA98 and TA100 (reverse mutation) (Ball *et al.*, 1988; Consolo *et al.*, 1989; Squadrito *et al.*, 1990; Shane *et al.*, 1991; Tokiwa *et al.*, 1993) and *Salmonella typhimurium* TM677 (forward mutation) (Ball *et al.*, 1988). Mutagenicity was enhanced in the presence of nitroreductase and/or O-acetylase activity (Consolo *et al.*, 1989; Oda *et al.*, 1993), and ring hydroxylation may also play a role (Consolo *et al.*, 1989). 3-Nitrofluoranthene reacted with DNA in rabbit tracheal epithelial cells (Gallagher *et al.*, 1987) and was DNA reactive following enzymatic or chemical reduction (Dietrich *et al.*, 1988). Topinka *et al*. (1998) reported 3-nitrofluoranthene-derived DNA adducts in a rat hepatocytes culture, a Clara cell derived human lung tumor cell line and a Chinese hamster lung cell line.

The levels of 3-nitrofluoranthene in human lung tissue from lung carcinoma patients were not statistically different from the levels in control patients (Horikawa *et al.*, 1998). Lung carcinoma subjects with levels >35 pg/gm lung had an increased risk of decreased five-year survival compared to lung carcinoma subjects with <35 pg/gm when based on adjusted rates (2.9-fold, 95%CI=0.8-10 and 2.4-fold, 95% CI=0.7-8.7, respectively).

The International Agency for Research on Cancer (IARC, 1984) concluded the data were inadequate to evaluate the carcinogenicity of 3-nitrofluoranthene in experimental animals. In a later evaluation IARC concluded based on the overall evidence that 3-nitrofluoranthene was not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1987). The studies by Busby *et al.* (1989), Horikawa *et al.* (1991) and von Tungeln *et al*. (1999) were released subsequent to the IARC reviews. 3-Nitrofluoranthene bears structural similarity to dinitrofluoranthene compounds that are on the Proposition 65 list of chemicals known to the State to cause cancer. Other nitropolycyclic aromatic compounds have been classified by IARC (2002) as possibly carcinogenic in humans. Among these compounds, 2-nitrofluorene and 5-nitroacenapthene share a five-membered ring with 3-nitrofluoranthene.

There is a **HIGH** level of **concern over the extent of exposure** to 3-nitrofluoranthene. 3-Nitrofluoranthene is a product of environmental nitration of fluoranthene and has been measured in air particulates (Arey et al., 1988; Tokiwa et al., 1993), in the dichloromethane extract of air particulates (Tokiwa et al., 1993) and in lung extracts (Horikawa et al., 1998). Among eight ambient air particulate samples collected in Southern California, Arey et al. (1988) reported measurable levels of 3-nitrofluoranthene in two samples and very low (*i.e.*, not reliably quantifiable) levels in an additional two samples. 3-Nitrofluoranthene may be further nitrated to form dinitrofluroanthenes,

compounds that exhibit carcinogenic properties (Tokiwa et al., 1993). 3-Nitrofluoranthene is not produced or used in commercial quantities.

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CARCINOGENICITY DATA SUMMARY: ORLISTAT

Preliminary evaluation of carcinogenicity and exposure data

Orlistat ((S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[2S,3S)-3-hexyl-4-oxo-2-oxetanyl] methyl]-dodecyl ester; tetrahydroliastatin, trade name, Xenical; CAS No. 96829-58-2), **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. There is one Phase III clinical trial of the drug finding breast cancer, but further follow-up suggests the finding may be unreliable. No treatment-related tumors were observed in rodent cancer bioassays.

Orlistat is a lipase inhibitor for obesity management that acts by inhibiting the absorption of dietary fats. In a Phase III clinical trial of approximately 1000 patients treated for up to two years, a 5.9-fold increased risk in breast cancer was observed in women taking 360 milligram daily. When cases of breast cancer occurring within the first six months of the study were excluded from the analysis, the relative risk during the study period in the 360-milligram group was 3.6 (95% confidence interval excludes one). If the survey period is included in the analysis the relative risk drops to 2.6 and the 95% confidence interval includes one. Confounding and detection effects were ruled out based on a well-balanced study (all known risk factors balanced among the three treatment groups) and previous mammography. Testimony during U.S. Food and Drug Administration (U.S. FDA, 1998) hearings found several potential problems with the follow-up study including, (1) a comparison between women on one year of treatment versus two was not performed, (2) younger women enrolled in the study were not included in the follow-up, (3) no follow-up was performed for women in the Phase II clinical trial (N=652), 75% of whom were prescribed 360 milligrams or greater. It has been noted that follow-up of nearly 8,000 women for only a few years showed no increase in the incidence of breast cancer associated with orlistat treatment (Anonymous, 2002).

No carcinogenic responses were observed after oral administration of doses of roughly 10-fold the human dose, in a study sponsored by the drug manufacturer. Orlistat did not exhibit mutagenic activity in bacteria or in the mammalian V79 cell/HPRT test. There was no clastogenic activity *in vitro* towards peripheral human lymphocytes or towards cultured rat hepatocytes in an unscheduled DNA synthesis assay. Orlistat was not mutagenic in an *in vivo* mouse micronucleus test (PDR, 2000).

There is a **HIGH** level of **concern over the extent of exposure** to orlistat. In April 1999, the FDA approved orlistat for seriously overweight patients. The drug is recommended for use three times a day, for periods of up to a year or longer. Consumer demand for weight-loss drugs is high.

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CARCINOGENICITY DATA SUMMARY: OXYFLUORFEN (GOAL)

Preliminary evaluation of carcinogenicity and exposure data

Oxyfluorfen (CAS No. 42874-03-3) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. Small treatment related increases in hepatocellular tumors were observed in male mice, but not in female mice nor in rats of either sex. Genotoxicity studies were predominantly negative.

One set of 87-week studies in CD-1 mice (60/sex/group) fed oxyfluorfen at concentrations of 0, 0.3, 3, or 33 mg/kg-day (0, 2, 20, or 200 ppm) produced an increased incidence of hepatocellular carcinomas in male mice (2/47, 0/47, 3/46 and 7/55) but not in female mice (U.S. Environmental Protection Agency [U.S. EPA], 2001). No carcinogenic effects were observed in two-year studies with male and female Long Evans rats (50/sex/group) fed doses of 0, 2, 40, or 800 (gradually increased to 1600) ppm (U.S. EPA, 2001).

No human *in vivo* or *in vitro* genotoxicity data were identified. Genotoxicity studies utilizing oxyfluorfen (96-99.7% purity) which were negative include three Ames tests in *Salmonella typhimurium*, a bacterial DNA damage/repair assay in *Escherichia coli*, two mouse lymphoma assays, one mouse micronucleus test, a HGPRT mutation assay in CHO cells, a chromosome aberration assay in CHO cells and *in vivo* assays for cytogenetic abnormalities in mice and unscheduled DNA synthesis in rats (U.S. EPA, 2001). One positive Ames assay in *Salmonella typhimurium* strain TA 100 was reported at a high, insoluble dose (U.S. EPA, 2001). Three positive Ames tests, a positive mouse lymphoma assay, two negative *in vivo* rat cytogenetic studies, and two negative *in vitro* assays for unscheduled DNA synthesis in rat hepatocytes utilized preparations of oxyfluorfen of 71.4 to 73% purity (U.S. EPA, 2001).

There is a **HIGH** level of **concern over the extent of exposure** to oxyfluorfen, a common herbicide used to control broadleaf and grassy weeds. The California Department of Pesticide Regulation (CDPR, 2002) reported that agricultural and commercial use of oxyfluorfen in California was approximately 348,000 lbs in 2001. Oxyfluorfen is also a component of household herbicides.

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CARCINOGENICITY DATA SUMMARY: PYRIMETHAMINE (DARAPRIM)

Preliminary evaluation of carcinogenicity and exposure data

Pyrimethamine [Daraprim®; CAS No. 58-14-0] **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list.** The chemical was not observed to cause cancer in long term bioassays in both sexes of $B6C3F_1$ mice and Fischer 344 rats. There is some carcinogenicity concern, however, because lung tumors were reported in intraperitoneal studies in mice and the chemical is clastogenic. No data on long-term effects of human exposure to pyrimethamine were identified.

The National Cancer Institute evaluated the carcinogenicity of pyrimethamine in mice and rats, finding no statistically significant increases in the incidence of tumors (NCI, 1978). In these studies male and female $B6C3F_1$ mice (n =35 per sex per dose) received pyrimethamine in the diet at doses of 0, 500 or 1000 ppm for 78 weeks and were followed until 102-105 weeks. Male and female Fischer 344 rats (n =35 per sex per dose) received 0, 200 or 400 ppm in the diet for 78 weeks and were followed until 104 weeks. In intraperitoneal injection studies of pyrimethamine in male and female A/He mice, Stoner *et al.* (1973) reported a statistically significant increase ($p< 0.05$) in the incidence of lung tumors in the high dose group compared to control mice.

No reports of *in vivo* genotoxicity in humans were identified. Pyrimethamine induced dose dependent chromosomal aberrations *in vitro* in human peripheral blood lymphocytes (Egli and Erdogan, 1991). Rats exposed to pyrimethamine exhibited increased numbers of chromosome aberrations (PDR, 2002). Clastogenicity was observed in the rat bone marrow micronucleus test (Ono *et al*., 1997). Pyrimethamine has been shown to interact with chromosomal centromeres, inducing aneuploidy in cultured cells (Parry *et al*., 2002). The drug was positive in the L5178Y/TK+/- lymphoma assay (PDR, 2002). In the Ames assay, pyrimethamine was not mutagenic in the presence or absence of metabolic activation in *Salmonella typhimurium* strains TA98, TA100, or TA1535 or TA1537 (Haworth *et al*., 1983).

Pyrimethamine has been reviewed by the International Agency for Research on Cancer (IARC, 1977; 1987) and classified in Group 3, based on "no adequate data" in humans and "limited evidence" in animals.

There is **HIGH** level of **concern over the extent of exposure** to pyrimethamine. Pyrimethamine is a dihydrofolate reductase inhibitor that is used as an antiparasitic drug. As noted in the Physicians' Desk Reference (2002), it is indicated for the treatment of toxoplasmosis when used conjointly with a sulfonamide, since synergism exists with this combination. Toxoplasmosis is a serious complication of AIDS. The drug is also indicated for the treatment of acute malaria, with a sulfonamide (*e.g.*, sulfadoxine) to initiate transmission control and suppression of susceptible strains of plasmodia. Because malarial resistance to pyrimethamine is prevalent worldwide, the drug is not suitable as a prophylactic agent for travelers to most areas.

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CARCINOGENICITY DATA SUMMARY: TRIETHANOLAMINE

Preliminary evaluation of carcinogenicity and exposure data

Triethanolamine (CAS No. 102-71-6) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**. There is some concern based on a finding of increased hepatic tumors in female mice. There are no adequate epidemiology studies.

There are no adequate human data on which to evaluate the carcinogenicity of triethanolamine. The International Agency for Research on Cancer (IARC, 2000) reported on epidemiology studies of workers using metalworking fluids with ethanolamines as additives, with or without sodium nitrite. Small excesses at various sites were observed, but no results were given specifically in relation to triethanolamine exposure. IARC (2000) noted the difficulty in drawing conclusions regarding triethanolamine from such data.

The National Toxicology Program (NTP, 1999) conducted skin painting studies in male and female rats and mice. In male rats, renal tubule adenomas were increased in treated male rats. NTP determined that this provided equivocal evidence of carcinogenicity. The female rat study provided no evidence of carcinogenicity. The studies in male and female mice were considered inadequate due to *Helicobacter hepaticus* infection. In male mice, findings of hepatocellular adenoma, hepatoblastoma, and carcinoma were characterized as uncertain. The NTP initially characterized the increases in hepatocellular adenoma and carcinoma in female mice as some evidence of carcinogenicity, before finally characterizing the study in its final report as inadequate due to the *Helicobacter* infection. The NTP studies in male and female mice were repeated, and the findings are available in a draft report (NTP, 2003). Liver hemangiosarcoma was elevated in the mid-dose group of male mice, but no dose related trend was observed, including after adjusting for survival. The NTP (2003) characterized these findings in male mice as providing equivocal evidence of carcinogenicity. In female mice, hepatocellular adenoma was increased, as well as hepatocellular adenoma and carcinoma combined. However, hepatocarcinoma alone was not significantly elevated in females. The NTP (2003) characterized these findings in female mice as providing some evidence of carcinogenicity.

Tumors were not increased in drinking water studies in male and female mice (Konishi *et al.*, 1992) or rats (Maekawa *et al.*, 1986). In feeding studies in ICR-JCL mice, increases in malignant lymphoma were observed in females (Hoshino and Tanooka, 1978). IARC (2000) discounted this study by noting the lack of historical control data on lymphosarcoma for female ICR-JCL mice, and that the triethanolamine dietary mixture was heated, possibly producing degradation products. NTP (2003) noted that, in a chronic study of sodium nitrite in ICR mice, the rate of lymphoma and nonthymic leukemia in control females was comparable to that observed in triethanolamine-treated females in the Hoshino and Tanooka study. Carcinogenic effects were not observed in male mice treated dermally with triethanolamine for 14 to 18 months (Kostrodymova *et al*., 1976, as cited by NTP, 2003). In a Tg.AC transgenic mouse model, dermal application produced no increase in tumors (Spalding *et al*., 2000).

Concerns have been raised regarding the potential endogenous formation of the carcinogen N-nitrosodiethanolamine following exposure to triethanolamine. The chemical was nominated for testing by the NTP based on this concern (NTP, 1999). IARC (2000) stated that there is no evidence that this conversion occurs under physiological conditions. Triethanolamine is structurally related to diethanolamine, which produces liver tumors in male and female mice. Genotoxicity assays of triethanolamine are generally negative (IARC, 2000; NTP, 1999; NTP 2003). Triethanolamine caused a significantly increased number of chromosome aberrations and a tendency to an increased frequency of sister chromatid exchanges in human lymphocytes *in vitro* according to a summary report by the Nordic Chemicals Group (Gillner and Loeper, 1993).

IARC (2000) classified triethanolamine as Group 3 (not classifiable) based on inadequate evidence in humans and animals.

There is a **HIGH** level of **concern over the extent of exposure** to triethanolamine. The chemical is widely used in consumer products and is a chemical intermediate. It is a registered pesticide in California, with 10,258 pounds of triethanolamine used in agricultural and commercial applications in 2001 (CDPR, 2002).

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CARCINOGENICITY DATA SUMMARY: VITAMIN K (BY INTRAMUSCULAR INJECTION IN NEONATES)

Preliminary evaluation of carcinogenicity and exposure data

Vitamin K [CAS No. 12001-79-5] administered to neonates by intramuscular injection **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list,** because the epidemiological data are predominantly negative, and there are no animal bioassay data. Vitamin K increased sister chromatid exchanges (SCE) in fetal sheep and in cultured human lymphocytes isolated from placenta and adult blood.

The International Agency for Research on Cancer (IARC, 2000) reviewed a series of epidemiological studies on the association between prophylactic vitamin K injection or oral administration in neonates and cancer. In none of the studies was cancer following oral administration of vitamin K found to be in excess of untreated neonates. A study by Golding *et al*. (1992) concluded that there was a significant excess of cancer (all types) in children who received prophylactic vitamin K injections as neonates (odds ratio 1.97, 95% confidence interval 1.3 to 3.0). In a study of 1,384,424 full term infants born after non-instrumental deliveries, Ekelund *et al*. (1993) reported odds ratios of 1.01 (95% C.I. = 0.88 to 1.17) for all childhood cancers and 0.90 (0.70 to 1.16) for childhood leukemia associated with prophylactic vitamin K injections. Kelbanoff *et al*. (1993) reported odds ratios of 0.47 (0.14 to 1.55) for leukemia and 1.08 (0.45 to 2.45) for all other cancers. Similarly, von Kries *et al*. (1996) reported odds ratios of 1.04 (0.74 to 1.48) for all childhood cancers and 0.98 (0.64 to 1.50) for leukemia associated with intramuscular vitamin K. Passmore *et al*. (1998) reported an increased risk of leukemia (odds ratio 1.44, $p = 0.05$) associated with vitamin K injections; however, they noted an effect of abnormal delivery, which could explain some of the findings. Based on these data IARC (2000) concluded there is *inadequate evidence* in humans for the carcinogenicity of vitamin K substances. Roman *et al*. (2002) conducted a pooled analysis of individual patient data from six case-control studies conducted in Great Britain and Germany to investigate the hypothesis that neonates who receive intramuscular vitamin K are at an increased risk of developing cancer, particularly leukemia. They found no convincing evidence that intramuscular vitamin K is associated with childhood leukemia.

No animal studies on the carcinogenicity of vitamin K were identified. On the basis of the human evidence and lack of animal evidence IARC (2000) concluded that Vitamin K substances are *not classifiable as to their carcinogenicity to humans*.

A single report (Israels *et al*., 1987) investigated the role of vitamin K on the induction of sister chromatid exchanges (SCEs) in cultured human lymphocytes from placental or adult blood. The mean number of SCEs per metaphase was significantly higher for cells treated with vitamin K for both adult and placental cells. This study also reported the effect of 1 mg of vitamin K administered by intravenous injection to fetal sheep. The SCE increased from $3.94 \pm$ S.E. 0.15 preinjection to $5.38 \pm$ S.E. 0.23 at 24 hr post-injection ($p<0.01$). Vitamin K1 did not induce mutations in *Salmonella typhimurium* (IARC, 2000).

There is a **HIGH** level of **concern over the extent of exposure** to vitamin K by intramuscular injection as it is routinely administered to almost all neonates in the U.S. Vitamin K is routinely administered prophylactically to neonates to prevent classical vitamin K deficiency bleeding.

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CARCINOGENICITY DATA SUMMARY: DIMETHIPIN (HARVADE)

Preliminary evaluation of carcinogenicity and exposure data

Dimethipin (2,3-dihydro-5,6-dimethyl-1,4-dithiin 1,1,4,4-tetraoxide; Harvade; CAS No. 55290-64-7) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, although there is some concern. In dietary studies, increases in pulmonary tumors were observed in female mice and in liver tumors in male rats.

Groups of CD-1 mice (50/sex/dose group) were fed diets containing concentrations of 0, 80, 400, and 2000 ppm dimethipin for 18 months (Hazleton Laboratories, 1981; as cited by U.S. EPA, 1993). The U.S. Environmental Protection Agency (U.S. EPA, 1990) summarized incidence data from the study. Significant increases in pulmonary carcinomas in the male mice fed 2000 ppm were observed relative to controls (1/49, 3/50, 3/50, 7/50 [Fisher's exact test, $p<0.05$]). A significant positive dose-related trend was reported for combined adenomas and carcinomas of the lung (6/49, 6/50, 6/50, 12/50; exact positive trend test, $p < 0.05$). When the Environmental Pathology Laboratories (EPL) re-evaluated the data, a marginal dose-related trend was indicated for pulmonary carcinomas, but the pairwise comparison was not significant (1/49, 1/49, 3/50, 4/50; exact positive trend test, $p = 0.067$). The combined incidence at the high dose was still significant in EPL's reanalysis (6/49, 7/49, 6/50, 14/50 [Fisher's exact test, $p<0.05$]). U.S. EPA indicated that the highest dose did not reach the maximum tolerated dose (MTD). No significant tumor findings among male mice were reported.

Groups of Sprague-Dawley rats (50/sex/group) were fed diets containing concentrations of 0, 40, 200, and 1000 ppm dimethipin for two years (Hazleton Laboratories, 1981; as cited by U.S. EPA, 1993). U.S. EPA (1990) reported the incidence data for the study. The pathological examination of liver tumors by Hazleton Laboratories and EPL provided different results. Hazleton Laboratories pathologists found a statistically significant increase in combined neoplastic nodules and carcinomas combined at the mid-dose group of male rats (3/50, 3/50, 10/50 [Fisher's exact test, $p < 0.05$], 4/50). In the reanalysis, EPL found a significant increase in neoplastic nodules in the 1000 ppm male rats (2/48, 2/48, 5/46, 8/42 [Fisher's exact test, $p < 0.05$]) and a significant positive dose-related trend in neoplastic nodules combined with carcinomas in the male rats (4/50, 3/49, 9/50, 10/50; exact positive trend test, $p < 0.05$). In a third analysis by a pathologist hired by Hazleton Laboratories, the incidence of neoplastic nodules was significant only at the mid-dose (0/50, 2/50, 7/50 [Fisher's exact test, $p<0.05$], 3/50); the same pattern was reported for combined neoplastic nodules and carcinomas (2/50, 3/50, 8/50 [Fisher's exact test, $p<0.05$], 5/50). U.S. EPA (1993) did not consider the Hazelton Laboratories historical control data useful for evaluation of the concurrent controls because of "differences in protocol (*e.g.*, route of administration, housing conditions, source of animals) across the several studies." No significant tumor findings were reported for female rats.

The California Department of Pesticide Regulation (CDPR, 2001) summarized an additional bioassay in Crl:CD® BR (VAF/plus) (Sprague-Dawley) rats conducted by MPI Research in 1996. Dimethipin was administered in the diet for two years at levels of 0, 40, 1750, or 3500 ppm to males (60/group) and 0, 40, 875, or 1750 ppm to females (60/group). Ten per sex per group were sacrificed at 12 months. Survival in high dose females was 65% of survival in the control group. No carcinogenic effects were reported.

U.S. EPA (1993) has classified dimethipin as a possible human carcinogen (Group C).

Dimethipin was weakly positive in a mouse lymphoma (L5178Y) assay in the presence of metabolic activation, and showed negative results in two assays for mutagenicity in *Salmonella* and in a sister chromatid exchange assay using Chinese hamster ovary (CHO) cells (U.S. EPA, 1993). Dimethipin did not induce micronuclei in CD-1 Swiss mice and did not induce unscheduled DNA synthesis in an *in vivo/in vitro* study in Wistar rats (FAO/WHO, 1989). CDPR (2001) summarized an *in vitro* study of chromosome aberrations in CHO cells, which was reported to be negative. The study was considered unacceptable by CDPR due to test article purity and stability and lack of individual data. Dimethipin did not induce mitotic gene conversion in *Saccharomyces cerevisiae* strain D4 (CDPR, 2001). Dimethipin also failed to induce mitotic aneuploidy in *Saccharomyces cerevisiae* strain D6, but this study was considered unacceptable due to lack of information on the purity of the compound and the use of triplicate plates in a single trial (CDPR, 2001).

There is a **MEDIUM** level of **concern over the extent of exposure to dimethipin** in California. Dimethipin is registered for use in California as a growth regulator. CDPR reported that approximately 746 pounds were applied to cotton in California in the year 2001 (CDPR, 2002).

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CARCINOGENICITY DATA SUMMARY: MECOPROP AND ITS SALTS[1]

Preliminary evaluation of carcinogenicity and exposure data

Mecoprop (MCPP; 2-[4-chloro-2-methylphenoxy]propanoic acid; CAS No. 7085-19-0) **and its salts did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, although there is some concern. This concern is based on observations of increased lung tumors in male mice in one of two studies, increased liver tumors in female mice in one of two studies, and limited evidence from human studies.

Chlorophenoxy herbicides have been investigated in a number of human studies, in which mecoprop was one of the exposures. Increases in soft-tissue sarcoma, lymphoma, nasopharyngeal cancer, colon and liver cancer have been associated with exposure to chlorophenoxy herbicides in some studies; however, interpretation of these results is confounded by exposures to multiple herbicides, and by the presence of chlorinated dioxins as contaminants of some chlorophenoxy herbicides (IARC, 1986 and 1987). The International Agency for Research on Cancer (IARC) determined in 1987 that chlorophenoxy herbicides were possibly carcinogenic to humans (Group 2B) and concluded that the evidence of carcinogenicity in humans was *limited* for chlorophenoxy herbicides. Since the IARC review, a population-based case-control study of four types of cancer and specific pesticides was conducted in six provinces in Canada. While the results for non-Hodgkin's lymphoma (NHL) were published in 2001 (McDuffie *et al.*, 2001), the results for other cancer sites (soft tissue sarcoma, Hodgkin's disease, and multiple myeloma) have not been published as of this writing. McDuffie *et al*. (2001) reported an odds ratio for NHL and mecoprop (10+ hours per year versus <10 hours) of 2.22 (95% CI 1.49-3.29) after adjustment for potentially confounding variables and other pesticides. The investigators did not explain whether the "exposed" designation (a rate of 10+ hours per year) was based on the subjects' lifetimes, working years, or any single year. The study was further hampered by potential bias from low questionnaire response rates (67% in the cases and 48% in the controls) and by multiple comparisons that could have caused significant results due to chance. There was no evidence of a dose-response relationship (OR = 2.27 for >0-2 days/year exposure versus OR= 2.06 for 2+ days/year).

The California Department of Pesticide Regulation (CDPR, 2000) summarized animal bioassays for various forms of MCPP[2]. BASF (1988; as cited by CDPR, 2000) exposed Wistar rats (75/sex/group) to MCPP (92.7% purity) via diet at levels of 0, 20, 100, or 400 ppm for 24 months. Ten rats per sex per group were sacrificed at 12 months. Oncogenic effects were not observed.

BASF (1986; as cited by CDPR, 2000) exposed B6C3F_1/CrlBR mice (50/sex/group) to MCPP-P (92.7% purity) in the diet at levels of 0, 25, 250, or 2500 ppm for 18 months. The high dose group exhibited severe effects on body weight at 11-12 months, and was discarded. CDPR (2000; 1999) reported that carcinomas of the lung were significantly increased in males in the 250 ppm group (0/49, 3/50, 5/50 [Fisher's exact test, $p < 0.05$]). No increases were seen in lung adenoma (5/49, 5/50, 3/50).

Follow-up studies were conducted by BASF (1999; as cited by CDPR, 2000) in which groups of 50 B6C3F_1/CrlBR mice were exposed to MCPP-P (92.7% purity) in the diet at levels of 0, 700 ppm (males), or 800 ppm (females) for 18 months. Clinical effects of the treatment were not observed. Carcinogenic effects were not observed in the lung in either sex. Hepatocellular adenoma (5/50; Fisher's exact test, $p < 0.05$) and carcinoma (4/50) were observed in treated females *versus* none in controls (0/50). Combined data were not presented.

CDPR (2000) summarized the genotoxicity data for MCPP. Negative results were reported in Ames assays with and without metabolic activation, an *in vivo* micronucleus test in mice, the CHO/HGPRT locus assay with and without metabolic activation, and an *in vitro* UDS assay in rat hepatocytes. CDPR (2000) discussed a series of studies of chromosome effects that showed mixed results. A study of Chinese hamsters exposed to MCPP (racemic mixture;

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[1] Methoxone sodium has been used in the past as a trade name for mecoprop, but this trade name is not currently used in the United States. There is information suggesting that methoxone sodium has been used in other countries as the trade name for a different compound. Given the above, the parenthetical "(including methoxone sodium)" has been deleted from the chemical name "mecoprop and its salts."

[2] According to CDPR (2000a), MCPP refers to the racemic mixture, MCPP-p or MCPP-P is the optically active (right-hand) isomer, MCPP-D is another name for the optically active (right-hand) isomer, and MCPP-pDMAS is the dimethylamine salt.

92.7% purity) showed a "possible increased frequency of chromosomal aberrations" in bone marrow. A similar study of MCPP-D did not detect chromosomal aberrations. One study of MCPP-P detected an increased frequency of chromosomal aberrations in human lymphocytes *in vitro* in the absence of activation; a second trial did not confirm the effect. A study of MCPP-pDMAS (dimethylamine salt) found an increased incidence of chromosomal aberrations at "a cytotoxic dosing level of 2500 µg/ml (highest concentration scored with activation)" in human lymphocytes *in vitro*. A "slight increase" in sister chromatid exchanges was observed at the two highest doses in bone marrow sampled from Chinese hamsters exposed to MCPP.

There is a **MEDIUM** level of **concern over the extent of exposure** to MCPP. MCPP is a chlorophenoxy herbicide primarily used on lawns and sport turf. Pounds of agricultural and commercial use in California for the different forms of MCPP were summarized by CDPR (2002):

MCPP	579 pounds
MCPP, potassium salt	3149 pounds
MCPP, dimethylamine salt	9687 pounds
MCPP-P, dimethylamine salt	641 pounds

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CARCINOGENICITY DATA SUMMARY: TRALKOXYDIM

Preliminary evaluation of carcinogenicity and exposure data

Tralkoxydim (CAS No. 87820-88-0**) did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, although there is some concern based on induction of benign testicular tumors in rats.

The California Department of Pesticide Regulation (CDPR, 2000) and the U.S. Environmental Protection Agency (U.S. EPA, 1998a) discussed studies in rats conducted by Zeneca in 1996. Groups of 52 male and female Alpk:APfSD rats were exposed to levels of 0, 50, 500 or 2500 ppm tralkoxydim in the diet for two years. Decreased body weight and food consumption were observed at the highest dose. Survival of high dose males was improved compared to controls or low dose males; this was attributed to a "reduction of deaths attributed to chronic progressive glomuleronephropathy." Benign Leydig cell testicular tumors were observed in all groups, showing a statistically significant trend and a statistically significant increase in the highest dose group.

One set of chronic studies was available in Syrian hamsters. CDPR (2000) stated, "Syrian hamster was used for the second rodent species due to a characteristic liver porphyria in tralkoxydim-treated mice…which would have limited the dose range in this species." Zeneca (1994; as cited by CDPR, 2000) exposed male and female Syrian hamsters to tralkoxydim at levels of 0, 250, 2500 or 7500 ppm in the diet for "up to 79 weeks." The study was considered inadequate due to very high mortality at study termination in all female groups (U.S. EPA, 1998a).

U.S. EPA (1998a) concluded that tralkoxydim is a "likely human carcinogen" based on the following: 1) The occurrence of rat benign Leydig cell tumors at all dose levels (the incidence in the high dose group exceeded the concurrent and historical control incidences); 2) the lack of an acceptable carcinogenicity study in a second species; and 3) the relevance of the testicular tumors to human exposure cannot be discounted.

U.S. EPA (1998b) and CDPR (2000) reported negative results for tralkoxydim in assays for gene mutation in bacteria, forward gene mutation in mouse lymphoma cells in culture, chromosome damage in human lymphocyte cells, unscheduled DNA synthesis in rat hepatocytes *in vivo*, and chromosome damage in mouse micronuclei *in vivo*.

There is a **MEDIUM** level of **concern over the extent of exposure** to tralkoxydim. Tralkoxydim is registered for use as an herbicide for grain crops in California. CDPR (2002) reported that eight pounds were applied to wheat in California in the year 2001. Because tralkoxydim is a newly registered active ingredient, use may increase over time.

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CARCINOGENICITY DATA SUMMARY: TRIFLUSULFURON-METHYL

Preliminary evaluation of carcinogenicity and exposure data

Triflusulfuron-methyl (CAS No. 126535-15-7) **did not reach a level of carcinogenic concern sufficient to be placed on the candidate list**, although there is some concern based on induction of benign testicular tumors in rats.

The California Department of Pesticide Regulation (CDPR, 1995) summarized the toxicology data available on triflusulfuron-methyl. Biegel (1993; as cited by CDPR, 1995) administered triflusulfuron-methyl in the diet at 0, 10, 100, 750, or 1500 ppm to male and female rats (strain not specified) for two years. A statistically significant increase in Leydig cell adenoma was observed in high dose males. No tumorigenic effects were observed in females. Biegel (1993; as cited by CDPR, 1995) administered triflusulfuron-methyl in the diet at 0, 10, 150, 2500, or 7000 ppm to male and female mice (strain not specified) for eighteen months. A statistically significant increase in the incidence of hepatocellular adenomas and carcinomas was observed in high dose male mice, but CDPR (1995) reported that the increase was within historical control levels for the laboratory. The U.S. Environmental Protection Agency (U.S. EPA, 2001) reported that male mice had statistically significant positive trends for hepatocellular carcinoma/adenoma combined. According to U.S. EPA, the increase was driven by the adenomas. Because the increases were not significant by pairwise comparison they were determined not to be carcinogenic effects by the U.S. EPA's Carcinogenicity Peer Review Committee. However, U.S. EPA (2002) cited this finding as possible evidence of carcinogenicity. U.S. EPA (1996) classified triflusulfuron-methyl as a Group C carcinogen based on a finding of testicular interstitial cell adenoma in CD-1 male rats.

Negative results were reported for mutagenicity assays in *Salmonella typhimurium* with and without metabolic activation, *in vitro* cytogenetic tests using Chinese hamster ovary cells with and without metabolic activation, the unscheduled DNA synthesis assay in cultured primary rat hepatocytes, and micronuclei induction *in vivo* using mouse bone marrow cells (CDPR, 1995). U.S. EPA (2001) noted that two assays for chromosomal aberrations in human lymphocytes were positive in the presence of metabolic activation. Inconclusive results were obtained in these assays in the absence of metabolic activation. du Pont (2001) reported that two of three *in vitro* assays for chromosomal aberrations were positive. du Pont stated that the positive results were obtained at high test concentrations where the solubility of the compound was considered "questionable."

There is a **MEDIUM** level of **concern over the extent of exposure** to triflusulfuron-methyl. Triflusulfuron-methyl is registered for use as an herbicide in California. One active product used on sugarbeets and in "tank mixes" was identified. According to the California Department of Pesticide Registration (CDPR, 2002), approximately 400 pounds of triflusulfuron-methyl were applied in California in 2001.

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CARCINOGENICITY DATA SUMMARY: INDOLIDAN

Preliminary evaluation of carcinogenicity and exposure data

Indolidan (1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyidozinyl)-2H-indol-2-one; CAS No. 100643-96-7) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list.** There is some concern for carcinogenicity, as the chemical caused benign adrenal medullary pheochromocytoma in rodents. Medullary hyperplasia was also observed.

Male Fischer 344 rats had increased benign adrenal medullary pheochromocytomas and medullary hyperplasia in the three dose groups tested (Sandusky *et al.*, 1991). Benign adrenal medullary pheochromocytomas were also increased among the high dose females, as was medullary hyperplasia in the three female treatment groups. The benign pheochromocytomas consisted of medullary cells that compressed the surrounding parenchyma, whereas the malignant pheochromocytomas observed (which did not alone reach statistically significance) consisted of medullary cells that were "less differentiated and invaded the cortex and surrounding tissues." A histochemical study of the indolidan-induced pheochromocytomas described above suggested the presence of a continuum between hyperplastic and neoplastic nodules (Tischler *et al.*, 1990).

In the only genotoxicity study identified by OEHHA, indolidan was not mutagenic towards *Salmonella typhimurium* (Rexroat *et al.*, 1995).

There is a **LOW** level of **concern over the extent of exposure** to indolidan. The chemical is a phosphodiesterase inhibitor that increases myocardial contractility (Sandusky *et al.*, 1991). However, indolidan shows signs of arrhythmogenic activity in patients with congestive heart failure (Corder *et al.*, 1992). It has not been approved for use as a drug in the United States (U.S. FDA, 2002), and a search of the U.S. Food and Drug Administration website failed to locate information on this drug. A review of the literature lead to the conclusion that indolidan is used primarily as a research chemical.

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CARCINOGENICITY DATA SUMMARY: ISOMAZOLE AND ISOMAZOLE HYDROCHLORIDE

Preliminary evaluation of carcinogenicity and exposure data

Isomazole (CAS No. 86315-52-8) **and isomazole hydrochloride** (2-[(2-methoxy-4-methylsulfinyl)-phenyl]-1H-imidazo[4,5-c]pyridine hydrochloride; CAS No. 87359-33-9) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list,** but there is some concern for carcinogenicity. In male rats, isomazole induced benign and malignant adrenal medullary pheochromocytomas; medullary hyperplasia was also observed. In female rats, there was a dose related increase in benign adrenal medullary pheochromocytomas (Sandusky *et al.*, 1991).

No human carcinogenicity data were identified.

Isomazole was not mutagenic towards *Salmonella typhimurium* (Rexroat *et al.*, 1995). In healthy humans (males), isomazole is rapidly absorbed and metabolized, and the metabolism appears to be self-induced (Woodworth *et al.*, 1991a; Woodworth *et al.*, 1991b).

There is a **LOW** level of **concern over the extent of exposure** to isomazole and isomazole hydrochloride. Isomazole is a pharmaceutical phosphodiesterase inhibitor that increases myocardial contractility (Sandusky *et al.*, 1991). Isomazole has not been approved for use as a drug in the U.S. (U.S. FDA, 2002). A review of the literature led to the conclusion that isomazole is used primarily as a research chemical.

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CARCINOGENICITY DATA SUMMARY: ACETOXYMETHYLPHENYLNITROSAMINE

Preliminary evaluation of carcinogenicity and exposure data

Acetoxymethylphenylnitrosamine (CAS No. 81943-37-5) **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list.** There is insufficient evidence in animal studies and no human epidemiologic data. One short-term (4-week), multi-dose subcutaneous injection study in groups of male and female hamsters (15 each) found increased malignant tumors at the site of injection ($p<0.04$) (Gold and Salmasi, 1982). Most tumors occurred at the low dose and a dose response pattern was not observed. Malignant tumors were not observed at the high dose, with the exception of one leukemia in a high-dose male. The survival times of both treated and untreated animals were poor and the total number of animals at risk was low. Therefore, this is an inadequate study of the carcinogenic potential of the compound.

Acetoxymethylphenylnitrosamine was found to be mutagenic in the *Salmonella typhimurium* strain TA1537 without enzymatic activation (CCRIS, 1994; Gold and Salmasi, 1982).

There is **NO IDENTIFIED CONCERN** over exposure to acetoxymethylphenylnitrosamine, as the only known use of this compound is as a research chemical, and it is not known to occur naturally.

References

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CARCINOGENICITY DATA SUMMARY: 1-BENZOYL-2,6-DIMETHYL-4-NITROSOPIPERAZINE

Preliminary evaluation of carcinogenicity and exposure data

1-Benzoyl-2,6-dimethyl-4-nitrosopiperazine [4-benzoyl-3,5,-dimethyl-nitrosopiperazine; CAS No. 61034-40-0] **did not reach a level of carcinogenicity concern sufficient to be placed on the candidate list**, but there is some carcinogenicity concern. This concern is primarily associated with observations in a long-term study in female rats (Singer *et al.*, 1981), which shows the development of benign and malignant tumors of the forestomach ($p=0.004$) and liver ($p<0.05$) relative to controls. This is the only long-term study of 1-benzoyl-2,6-dimethyl-4-nitrosopiperazine that has been conducted to date. The cancer study employed only one treated dose group (Singer *et al.*, 1981).

Carcinogenicity concern is tempered by the fact that 1-benzoyl-2,6-dimethyl-4-nitrosopiperazine did not cause mutations in yeast (*Saccharomyces cerevisiae*) (Larimer *et al.*, 1980) or in bacteria (*Salmonella typhimurium*, strains TA98, TA100, TA1535 or TA1537) (Rao *et al.*, 1978; Lijinsky and Andrews, 1983). The genotoxicity evaluation by U.S. EPA's GENE-TOX program was inconclusive (GENE-TOX, 1995; Kier *et al.*, 1986; Rao *et al.*, 1978).

Carcinogenicity concern regarding 1-benzoyl-2,6-dimethyl-4-nitrosopiperazine is also tempered by structure-activity information. Other nitroso-piperazine derivatives have been tested for genotoxic and carcinogenic potential. Some nitroso-piperazine derivatives, namely N-nitroso 3,5-dimethylpiperazine, N-nitroso-3,4,5-trimethylpiperazine, dinitrosopiperazine, 2-methyl-dinitrosopiperazine, 2,5-dimethyl-dintrosopiperazine, 2,6-dimethyl-dintrosopiperazine and dinitrosohomopiperazine, were strongly carcinogenic in rats (reviewed in Rao *et al.*, 1978, Singer *et al.*, 1981). All of these cyclic nitroso compounds were mutagenic in bacteria (Rao *et al.*, 1978; Lijinsky and Andrews, 1983). Compounds that were not mutagenic, such as 2,3,5,6-tetramethyl-dinitrosopiperazine, were not carcinogenic in rats. However, two derivatives exhibited some mutagenic activity but were not carcinogenic when tested in the rat (Rao *et al.*, 1978).

There is **NO IDENTIFIED CONCERN over the extent of exposure** to 1-benzoyl-2,6-dimethyl-4-nitroso-piperazine. No exposure information was located for this compound. It appears to be a laboratory compound used by Singer *et al*. (1981) to study the structure activity of nitroso-piperazine derivatives.

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CARCINOGENICITY DATA SUMMARY: PIMOZIDE

Preliminary evaluation of carcinogenicity and exposure data

There are **inadequate data to assign a level of carcinogenicity concern to pimozide** [Orap®; 1-[1-[4,4-bis(4-fluorophenyl)butyl]-4-piperidinyl]-1,3-dihydro-2H benzimidazole-2-one; CAS No. 2062-78-4]. The only information available comes from a drug label produced by a manufacturer, Teva Pharmaceuticals USA. According to information on the label, pimozide caused an increase in the incidence of benign pituitary tumors and mammary tumors in female mice treated for 18 months at doses 15 times the recommended human dose, on a mg/kg basis (Teva Pharmaceuticals USA, 1999). The label also cites a negative bioassay in rats treated for 24 months at doses up to 50 times human dose on mg/kg basis, but notes that survival was poor. Specific data on the type of tumors (benign/malignant), incidences, number and size of treatment groups were not provided and preclude determining the level of carcinogenicity concern for this compound. Other chemicals in this class (neuroleptics/antipsychotics) have shown evidence of mammary tumorigenesis. Mammary effects have been proposed to result from hyperprolactimenia, due to the anti-dopaminergic effects of this chemical on the pituitary. The drug label also noted that hyperprolactinemia has been observed in humans treated with anti-dopaminergic antipsychotic agents.

Other relevant data include negative findings for reverse mutations in four *Salmonella* strains, dominant lethal mutations in mice, and in a micronucleus test in rats (Teva Pharmaceuticals USA, 1999; Balbi *et al.*, 1980).

Under the Warnings section of the drug label, it is stated that "ORAP may have tumorigenic potential. Based on studies in mice, it is known that pimozide can produce a dose-related increase in pituitary tumors. The full significance of this finding is not known, but should be taken into consideration in the physician's and patient's decisions to use this drug product. This finding should be given special consideration when the patient is young and chronic use of pimozide is anticipated" (Teva Pharmaceuticals USA, 1999).

There is a **HIGH** level of **concern over the extent of exposure** to pimozide. Pimozide is a pharmaceutical antipsychotic agent, used in the treatment of motor and phonic tics in patients with Tourette Syndrome (Teva Pharmaceuticals USA, 1999). It has been estimated that the number of those with Tourette Syndrome in the U.S. is 100,000 (Tourette Syndrome Association, Inc., 2002). While pimozide is not indicated as a treatment of first choice – it is prescribed when other medications fail – it is expected that there may be a number of patients who are treated at relatively high levels over a long period.

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