DRAFT PRIORITIZED CANDIDATE CHEMICALS UNDER CONSIDERATION FOR CARCINOGENICITY EVALUATION

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Chemicals were selected for prioritization from Category I of the tracking database by the process described in the document entitled "Procedure for Prioritizing Candidate Chemicals for Consideration Under Proposition 65 by the State's Qualified Experts" (May 1997). Three batches of ten chemicals each were selected in a first round pilot random selection from among 85 within Category I of the tracking database for which toxicity information had been entered into the toxicity field of the data entry sheet. For 28 of these 30 selected chemicals, draft data summaries were prepared and draft priorities assigned. Draft data summaries were not prepared for the remaining two chemicals, i.e., C.I. solvent yellow 14 and salicylazosulfapyridine, since these chemicals are currently candidates for listing as carcinogens under the authoritative bodies listing mechanism of Proposition 65 (See *California Regulatory Notice Register*, August 22, 1997). Prioritized chemicals with a final high level of carcinogenic hazard concern will be assigned to the Candidate List, from which chemicals will be chosen for the preparation of a hazard identification document. All other final prioritized chemicals will be assigned to Category II. 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.	Draft Level of Carcinogenicity Concern	Draft Level of Exposure Concern	Page
p-chloronitrobenzene	100-00-5	high	high	3
estragole	140-67-0	high	high	5
furfural	98-01-1	high	high	8
trichloroacetic acid	76-03-9	high	high	11
bis(2-chloro-1-methylethyl)ether	108-60-1	high	medium	15
2-aminofluorene	153-78-6	high	low	18
4-amino-2-nitrophenol	119-34-6	high	low	21
acronycine	7008-42-6	high	n. i. c.	23
2-amino-5-(5-nitro-2-furyl)-1,3,4-oxadiazole	3775-55-1	high	n. i. c.	25
N-butyl-N-nitrosourea	869-01-2	high	n. i. c.	27
2,5-dimethoxy-4'-aminostilbene	23435-31-6	high	n. i. c.	29
N-ethyl-N-formylhydrazine	74920-78-8	high	n. i. c.	31
chlorthal-dimethyl	1861-32-1	medium high	high	33
cyanazine	21725-46-2	medium high	high	35
dapsone	80-08-0	medium high	high	39
2,4-dichlorophenoxyacetic acid and its salts and esters	94-75-7	medium high	high	42
dimethyl hydrogen phosphite	868-85-9	medium high	high	47
malonaldehyde and its salts	24382-04-5	medium high	high	49
zearalenone	17924-92-4	medium high	high	52
2-biphenylamine (and strong acid salts)	2185-92-4	medium high	medium	55
bromoethane	74-96-4	medium high	medium	57
β-thioguanine deoxyriboside	789-61-7	medium high	n. i. c.	60
bis(tri-*n*-butyltin)oxide	56-35-9	medium	high	62
dicofol	115-32-2	medium	high	64
di(2-ethylhexyl)adipate	103-23-1	medium	high	67
patulin	149-29-1	medium	high	70
pentachloronitrobenzene	82-68-8	medium	high	73
piperonyl sulfoxide	120-62-7	medium	low	77
POSTPONED		**Status**		
salicylazosulfapyridine [a]	599-79-1	Authoritative body finding under evaluation by OEHHA		
C.I. solvent yellow 14 (Sudan 1) [a]	842-07-9	Authoritative body finding under evaluation by OEHHA		

[a] Potential listing *via* authoritative bodies provision (22 CCR § 12306)
n. i. c. = No Identified Concern

CARCINOGENICITY DATA SUMMARY: *P*-CHLORONITROBENZENE

p-Chloronitrobenzene (CAS No. 100-00-5) is an intermediate in the manufacture of pesticides (parathion), drugs (4-acetylaminophenol), dyes, lumber preservatives, and photographic chemicals. SRI (1994) estimated domestic production of *p*-chloronitrobenzene at about 145 million pounds. *p*-Chloronitrobenzene has been detected in samples of river water, groundwater and edible fish in the US (Howard, 1989). IARC (1996) classified this chemical as a group 3 chemical due to inadequate evidence in humans and experimental animals. US EPA (1995) classified *p*-chloronitrobenzene as a group B2 carcinogen indicating there is sufficient evidence of carcinogenicity in animals and insufficient or lack of evidence in humans.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Mice long-term feed studies: Weisburger *et al*., 1978. Groups of 25 CD-1 mice of each sex were fed diets containing p-chloronitrobenzene at three doses (0, 3,000 and 6,000 mg/kg diet) for 18 months. The mice were then held for three months on control diet prior to terminal examination. The incidence of vascular tumors was increased in high-dose males (controls, 0/14; low-dose mice, 2/14; high-dose mice, 4/14; $p<0.025$ versus concurrent and pooled control incidence of 5/99) and in high-dose females (controls, 0/15; low-dose mice, 2/20; high-dose mice, 7/18; $p<0.025$ versus concurrent and pooled control incidence of 9/102). The incidence of hepatocellular carcinoma was increased in low-dose males (controls, 1/14; low-dose mice, 4/14 ; $p<0.025$ versus pooled control incidence of 7/99. IARC (1996) considered the study inadequate due to the small number of animals and the limited histopathological evaluation and reporting.

2. Rat long-term feed studies: Weisburger *et al*., 1978. Groups of 25 male Charles River CD rats were fed diets containing p-chloronitrobenzene at 0, 2000 or 4000 mg/kg for 3 months. After that time, the dietary concentrations were lowered to 0, 250 and 500 mg/kg for two months, then raised to 0, 500 and 1000 mg/kg for 13 months; rats were then held for 6 months on control diet prior to terminal examination. No increase in tumor incidences were reported. IARC (1996) considered the study inadequate due to the small number of animals, the short duration of dosing, and the limited histopathological evaluation and reporting.

Other relevant data

p-Chloronitrobenzene induced reverse mutation but not primary DNA damage in bacteria. At toxic doses, it induced chromosomal aberrations, sister chromatid exchange and repairable DNA breaks in cultured mammalian cells. Intraperitoneal injection into mice induced DNA damage in the liver, kidney, and brain (IARC, 1996). In a US EPA (1985) report, it is indicated that rabbit can metabolize *p*-chloronitrobenzene into *p*-chloroaniline. *p*-Chloroaniline is listed as a 2B carcinogen by IARC (1993) and a cancer causing chemical under Proposition 65. Administration of *p*-chloroaniline is associated with increased incidences of uncommon sarcomas of the spleen in male F344/N rats and hepatocellular neoplasms and of hemangiosarcomas of the liver or spleen in male $B6C3F_1$ mice (NTP, 1989). Some bacteria in human intestinal tract have nitroreductases and can reduce *p*-chloronitrobenzene into *p*-chloroaniline (Raffi *et al*., 1995). Based on the results of a computerized analysis of structure-activity relationships using a set of rules generated by US EPA experts (Oncologic, version 2.5), it is predicted that *p*-chloronitrobenzene is of moderate concern.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** since *p*-chloronitrobenzene caused increased incidence of rare tumors, i.e., vascular tumors, in CD-1 mice of both sexes. This is reinforced by the genotoxic activity in a number of short-term tests and the fact that a metabolite, *p*-chloroaniline, is a carcinogen listed under Proposition 65. US EPA (1995) classified *p*-chloronitrobenzene as a group B2 carcinogen. However, IARC (1996) considered there is inadequate data in humans and experimental animals and classified the compound as a group 3 chemical.

There is **HIGH** level of **concern over the extent of exposure**. *p*-Chloronitrobenzene has a large production volume and is used in the manufacture of many important chemicals such as pesticides, drugs, dyes, and lumber preservatives. Although *p*-chloronitrobenzene is a solid at room temperature, its vapor pressure is sufficiently high to make inhalation exposure a concern. Also, based on animal and occupational exposure information, *p*-chloronitrobenzene may be readily absorbed through the skin. Because both inhalation and dermal routes of exposure are possible, occupational exposure is expected to be important. In addition, the general population may be exposed to this chemical through the ingestion of contaminated water and fish.

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CARCINOGENICITY DATA SUMMARY: ESTRAGOLE

Estragole (1-methoxy-4-(2-propenyl) benzene; esdragol; estragon; tarragon; CAS No. 140-67-0) is a major constituent of the oils of tarragon, sweet basil, fennel, Chinese star anise, Russian anise, and turpentine, is used as a food additive in spices and essential oils, and is used in perfumes (HSDB, 1997; Miller et. al., 1983). US production in 1981 was estimated to be greater than 9,000 kg; imports into the US in the same year were approximately 8,000 kg (HSDB, 1997). It is considered as GRAS (generally regarded as safe) by the FDA (1996).

Carcinogenicity Data available:

Epidemiological studies

No studies on the long-term health effects of human exposure to estragole have been reported. However, it is notable that many different groups of people worldwide consume large quantities of herbs containing estragole, with no known or suspected adverse health effects.

Animal bioassays

1. Newborn mouse subcutaneous (s.c.) injection study (4X on days 1, 8, 15, 22 observed for 15 months): Drinkwater *et al*., 1976. Male CD-1 mice were given a total dose of either 4.4 or 5.2 µmol estragole by s.c. injection in 4 doses on days 1, 8, 15, and 22. Significant increases in the incidences of hepatocellular carcinomas were observed in treated animals, as compared with controls (6/51 vehicle controls; 14/60 low-dose ($0.05 < p < 0.1$); 7/18 high-dose ($p < 0.2$)). Three lung adenomas and 3 malignant lymphomas were found in low-dose animals but the incidences of these tumors did not differ significantly from controls.

2. Newborn mouse gavage studies (2X/wk for 5 weeks, starting on day 4, observed for 14 months): Miller *et al*., 1983. Fifty-five male and 49 female newborn CD-1 mice were given 2.5 µmol estragole/g body weight in trioctanoin by stomach tube twice a week for 10 times. At 14 months, 73% ($p < 0.001$) of treated males, 9% of treated females, 24% of male solvent controls and 2% of female solvent controls developed hepatomas (types A or B).

3. Newborn mouse intraperitoneal (i.p.) injection study (4X on days 1, 8, 15, 22, observed for 12 months): Miller *et al*., 1983. Fifty-two male newborn CD-1 mice were injected i.p. with a total dose of 9.45 µmol estragole/g body weight in 4 doses at a ratio of 1:2:4:8 on days 1, 8, 15, and 22. At 12 months, hepatomas (types A or B) occurred in 65% of treated animals ($p < 0.001$, treated vs. solvent controls), in 26% of solvent (trioctanoin) controls, and in 15% of untreated controls.

4. Newborn mouse i.p. injection study (4X on days 1, 8, 15, 22, observed for 18 months): Miller *et al*., 1983. Forty-seven male B6C3F1 mice were injected i.p. with a total dose of 4.75 µmol estragole/mouse in 4 doses at a ratio of 1:2:4:12 on days 1, 8, 15, and 22. At 18 months, hepatomas (types A or B) occurred in 83% of treated animals ($p < 0.001$, treated vs. solvent controls), in 41% of solvent (trioctanoin) controls, and in 28% of untreated controls.

5. Mouse diet study (12 months treatment + 8 months observation): Miller *et al*., 1983. Groups of 50 female CD-1 mice were given 0.23% or 0.46% estragole in diet for 12 months and were examined at 20 months from the start of the experiment. Hepatomas (types A or B) occurred in 56% of low-dose animals ($p < 0.001$), in 71% of high-dose animals ($p < 0.001$), and in none of the untreated controls.

6. Preweanling mouse single i.p. injection study (day 12, then observed for 10 months): Wiseman *et al*., 1987. 0.75 µmol estragole dissolved in trioctanoin/g body weight was given to 40 male B6C3F1 mice in a single i.p. injection at 12 days of age. Fifty-nine vehicle controls received trioctanoin i.p. At the end of the 10 month observation period, 95% of the mice receiving estragole had hepatomas (types A or B) as compared with 17% of the corresponding controls ($p < 0.001$).

Other relevant data

In one study, estragole was not mutagenic in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 in the absence or presence of hamster or rat liver S9 (Zeiger *et al.*, 1987). In another study, estragole was not mutagenic in strains TA 98, TA 100, TA 1537, or TA1538 +/- metabolic activation, and was only weakly mutagenic in TA 1535 in the absence of metabolic activation (To *et al.*, 1982). Increased mutagenic activity was observed in strain TA1535 in the presence of rat liver S9 plus 3'-phosphadenosine-5'-phosphosulfate (PAPS) (To *et al.*, 1982). In another study, estragole was weakly positive in strain TA 100 and 1'-hydroxyestragole, the proximate carcinogenic metabolite of estragole, was clearly positive in TA 100 (Swanson *et al.*, 1979).

Estragole caused a marked induction of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* in two studies (Howes *et al.*, 1990; Muller *et al.*, 1994) and induced DNA repair in rat liver *ex vivo*, but it did not cause chromosomal aberrations in V79 cells (Muller *et al.*, 1994). Estragole binds to DNA (Randerath *et al.*, 1984), RNA, proteins, and other small molecules (Miller and Miller, 1983).

A single i.p. injection of 1 μmol estragole/g body weight did not result in the induction of lung tumors in female strain A/J mice (Miller *et al.*, 1983).

Other alkylbenzenes, like the propenyl analogues methyleugenol and safrole, and isosafrole are hepatocarcinogenic in experimental animals (Miller *et al.*, 1983). 1'-Hydroxyestragole, the proximate carcinogenic metabolite of estragole, induced significantly increased incidences of hepatomas in newborn male B6C3F1 mice when administered i.p. (Miller *et al.*, 1983) and in preweanling male C3H/HeJ mice when administered s.c. (Wiseman *et al.*, 1987).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over estragole based on the observation of liver tumors in several strains of male newborn or preweanling mice via several different routes of administration. The concern is reinforced by the observation that the treatment period was often relatively short (i.e., often spanning only 4-5 weeks) and the observation period was often less than lifetime (i.e., 10, 12, 14, or 15 months). The induction of UDS in rat hepatocytes and DNA repair in rat liver by estragole, combined with its ability to bind to DNA, RNA, and proteins considerably adds to the level of concern. In addition, the demonstrated carcinogenicities of 1'-hydroxyestragole, a metabolite of estragole, and of several structurally related compounds reinforces the level of carcinogenicity concern. An important consideration which moderates the level of concern somewhat is the observation that various groups of people living in disparate areas of the world consume large quantities of estragole in the diet, in the absence of any noticeable adverse health effects. This may suggest that the carcinogenic activity of estragole is either species-specific, or occurs only at high doses.

There a **HIGH** level of **concern over the extent of exposure** to estragole. It is found naturally in tarragon, sweet basil and other spices, and is used as a food additive in other spices and oils. Foods containing these spices or oils are consumed on a regular basis in California.

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CARCINOGENICITY DATA SUMMARY: FURFURAL

Furfural (2-furanaldehyde; 2-furancarbonal; furfurylaldehyde; formylfuran; CAS No. 98-01-1) is used as a chemical intermediate, a solvent, a constituent of rubber cements, a weed killer, a fungicide, and a flavoring agent. USEPA classifies furfural as a toxic waste according to specific use histories (HSDB, 1997). Furfural occurs naturally as a volatile component of some fruits and vegetables and is commercially produced from renewable agricultural sources (e.g. oat hulls and corn cobs) by acid hydrolysis. Between 1965 and 1989, 45,000- to 57,000 tons were used annually in the United States (IARC, 1995). In the USA, 135,016 workers were exposed to furfural in 1983 (NIOSH, 1983, reported in IARC, 1995). Occupational or environmental exposure to furfural may also occur during wildland fires. Furfural is apparently not used in California as a pesticide, since is not listed in the California 1995 Pesticide Use Report (DPR, 1995). Furfural was classified by IARC (1995) as a Group 3 carcinogen (not classifiable as to its carcinogenicity) based on inadequate evidence in humans and limited evidence in experimental animals.

Carcinogenicity data available:

Epidemiological studies

No data on long-term effects of human exposure to furfural were found in an earlier search by IARC (1995) or more recently by OEHHA.

Animal Studies

1. Rat long-term gavage study (103 weeks): NTP, 1990. Male and female Fischer 344 rats (50/sex/group) were administered 0, 30 or 60 mg/kg body weight furfural dissolved in corn oil by gavage, 5 days/week for 103 weeks. In high-dose (HD) males, 2/50 developed rarely occurring cholangiocarcinomas compared to 0/50 among controls ($p = 0.2$). Two/50 HD males exhibited biliary dysplasia with fibrosis compared to 0/50 among controls. The combined (4/40) cholangiocarcinomas and biliary dysplasia with fibrosis was marginally statistically significant ($p = 0.06$). The historical incidence of this tumor in control rats at the testing laboratory was 3/2145 ($p < 0.005$ for comparison of frank tumors with historical incidence). The evaluation by NTP (1990) of the significance of the cholangiocarcinomas plus biliary dysplasia with fibrosis follows. "The Pathology Working Group that reviewed the lesions considered the latter to be an early stage in the development of cholangiocarcinoma. .... "Cholangiocarcinomas are uncommon neoplasms in F322/N rats and have been observed in only 3/2145 corn oil vehicle control male rats in previous NTP 2-year studies. .... Since both cholangiocarcinomas and biliary dysplasia with fibrosis occurred in high dose male rats, their presence was judged to constitute some evidence of carcinogenic activity". NTP (1990) concluded furfural exhibited some evidence of carcinogenicity male rats, and no evidence of carcinogenicity in female rats.

2. Mouse long-term gavage study (103 weeks): NTP, 1990. Male and female B6C3F1 mice (50/sex/group) were administered 0, 50, 100 or 175 mg/kg body weight furfural dissolved in corn oil by gavage, 5 days/week for 103 weeks. In males, the incidences of hepatocellular adenomas increased with dose ($p = 0.004$, life table test; 9/50 controls, 13/50 low dose, 11/49 mid dose and 19/50 high dose) and were significantly greater in the high dose group compared to controls ($p = 0.004$, life table test). The incidences of hepatocellular carcinomas in males increased with dose ($p = 0.001$, life table test; 7/50 controls, 12/50 low dose, 6/49 mid dose and 21/50 high dose) and were also significantly increased in the high dose group compared to controls ($p < 0.001$, life table test). In females, the incidences of hepatocellular adenomas increased with dose ($p = 0.0008$, life table tests; 1/50 controls, 3/50 low dose, 5/50 mid dose and 8/50 high dose) and were significantly greater in the high dose group compared to controls ($p = 0.017$, life table tests). The combined incidences of hepatocellular adenomas and carcinomas were: in males, 16/50 controls, 22/50 low dose, 17/49 mid dose and 32/50 high dose (life table trend and high dose test p-value each < 0.001). Among females the combined incidences of hepatocellular adenomas and carcinomas were: 5/50 controls, 3/50 low dose, 7/50 mid dose, and 12/50 high dose (life table trend and high dose test p-values, 0.012 and 0.050 respectively). There was also a marginal increase in the incidence of forestomach papillomas in females at the high dose (6/50 vs. 1/50 in controls; $p = 0.058$). NTP (1990) concluded furfural exhibited clear evidence of carcinogenicity in male mice and some evidence of carcinogenicity in female mice.

3. Mouse dermal exposure study: Miyakawa *et al*., 1991. To investigate the initiating activity of furfural, female CD-1 mice (20/group) received topical applications of 50 μmol furfural dissolved in 0.1 dimethyl sulfoxide (DMSO) on the back twice a week for 5 weeks. One week after the last treatment, the mice were treated twice a week with 2.5 μg of the promoter 12-O-tetradecanoylphorbol 13-acetate (TPA) in 0.1 ml acetone for 47 weeks. One control group was treated with furfural and acetone, a second with DMSO and TPA, a third with DMSO and acetone and a fourth with a total dose of 100 μg 7,12-dimethylbenz[a]anthracene and TPA (positive control). Five of 19 mice given furfural and TPA developed 7 skin papillomas and 1 squamous-cell carcinoma, whereas only 1/20 mice given DMSO and TPA had a papilloma. According to the study authors, the data on furfural/TPA (compared to DMSO/TPA) was not statistically significant by Fisher's exact test. IARC (1995) reports $p = 0.08$ (Fisher's exact test). None of the negative controls developed tumors, but all 20 mice in the positive control group developed skin tumors. An analysis by the authors of the time-to-tumor data revealed a statistically significant increase in the incidence of skin tumors among the mice treated with furfural/TPA compared to mice treated with DMSO/TPA ($0.01 < p < 0.05$ by Peto's trend test. IARC (1995) and the authors concluded that furfural has weak tumor initiating activity in this two-stage assay.

4. Hamster inhalation study (52 weeks + 28 weeks observation): Feron and Kruysse, 1978. Male and female Syrian golden hamsters (30/sex/group) were exposed to 250 to 400 ppm furfural vapor, 7 hours/day, 5 days/week for 52 weeks. Two control groups (18/sex/group) exposed to air only or given weekly intratracheal instillations of 0.2 ml 0.9% NaCl solution were included. To test the co-carcinogenic effect of furfural, other groups (30/sex/group) were given benzo(a)pyrene (BP) intratracheally (0.2 ml 0.175% or 0.35% BP in 0.9% NaCl solution) on a weekly basis for 52 weeks or diethylnitrosamine (DENA) subcutaneously (0.2 ml 0.0625% DENA in 0.9% NaCl solution) once every 3 weeks for 52 weeks. The experiment was terminated at week 80 when all survivors were killed and autopsied. The authors concluded that furfural had no carcinogenic activity itself, or any enhancing effect on BP- or DENA- induced carcinogenesis in hamsters.

5. Hamster intratracheal instillation study (1dose/week for 36 weeks + 42 weeks observation): Feron, 1972. To determine if furfural is a complete carcinogen or a co-carcinogenic agent, male and female hamsters (35/sex/group) received 36 weekly intratracheal instillations of furfural, benzo(a)pyrene (BP), and BP plus furfural and were then observed for 78 weeks. The control group, consisting of 35 males, received 0.9% NaCl solution. There was no indication that furfural possessed carcinogenic activity itself. BP alone induced 41/62 respiratory tumors, mostly squamous-cell carcinomas. BP plus furfural resulted in earlier development of metaplastic changes of the tracheobronchial epithelium, a shorter latent period for tracheobronchial tumors, and a few more bronchial and peripheral squamous-cell carcinomas. The authors concluded furfural had a slight co-carcinogenic effect in the hamster respiratory tract.

Other relevant data

Furfural was not mutagenic in *S. typhimurium* or *E. coli*. Injection, but not feeding, of furfural to adult *Drosophila melanogaster* induced sex-linked recessive lethal mutation. Furfural did not induce heritable reciprocal translocations in *D. melanogaster*. Furfural was mutagenic (wing spot test for mutational activity in somatic cells) and clastogenic (Mei-9a test for chromosome loss in germ-line cells) in *D. melanogaster*. Furfural induced gene mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells in the absence of metabolic activation. It induced sister chromatid exchange in Chinese hamster ovary cells and human lymphocytes and chromosomal aberrations in Chinese hamster ovary and V79 lung cells in the absence of metabolic activation. The frequencies of sister chromatid exchange and chromosomal aberrations were not increased in the bone-marrow cells of B6C3F1 mice injected intraperitoneally with single doses of furfural up to 200 mg/kg_{bw} (NTP, 1990). Furfural reacts with DNA *in vitro*, primarily at base pairs, leading to destabilization of the secondary structure of DNA and to single-strand breaks. These studies and their results were reviewed by NTP (1990) and IARC (1995).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** for furfural based on the development of carcinogenicity in two sexes of one species (mouse). The decision by NTP (1990) to consider the rare cholangiocarcinoma plus bile duct dysplasia with fibrosis as some evidence for carcinogenicity in male rats, based on the evaluation of the Pathology Work Group, supports this level of concern. IARC (1995) classified furfural as a group 3 carcinogen (not

classifiable as to its carcinogenicity) in a document that included a review of the NTP (1990) data, however no discussion of the data or the evaluation were presented in the IARC (1995) publication. Results from an initiation-promotion assay on mouse skin that indicate furfural has weak initiating activity add to the level of concern. The level of concern is reinforced by results from short term *in vivo* and *in vitro* tests that show furfural is mutagenic and clastogenic and that it destabilizes secondary DNA structure.

There is a **HIGH** level of **exposure concern** for furfural. The general population may be exposed through the consumption of food containing furfural as an additive or as a natural component. The chemical is widely used and occupational and environmental exposure could occur through dermal and/or inhalation uptake. Among humans, dermal absorption of furfural is substantial (specific numbers not given) and pulmonary absorption is about 20-30 percent of the amount inhaled (NTP, 1990).

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CARCINOGENICITY DATA SUMMARY: TRICHLOROACETIC ACID

Trichloroacetic acid (TCA, CAS No. 76-03-9) has a number of industrial applications, including use as a synthetic intermediate, and various other minor uses, *e.g.* as a medication, and as a reagent for albumin detection. In addition to its deliberate production and uses it occurs (at levels up to 200 µg/l) as a contaminant of drinking water disinfected by chlorination (IARC, 1995). It is formed (along with other chloroacetic acids, halomethanes, and related compounds) by reaction of chlorine or hypochlorite with organic substances such as humic acid. It is also found in other situations where water is chlorinated, such as irrigation, swimming pools, and pulp mill effluents. Various other chlorinated compounds are also formed in the same way, and some of these are metabolized *in vivo* to TCA, resulting in additional exposure to this compound. Photodegradation of tetrachloroethylene in water also results in the formation of TCA (IARC, 1995). Some of these precursors of TCA also have major industrial uses (*e.g.* trichloroethylene and tetrachloroethylene).

Free TCA is a strong organic acid which forms water-soluble salts with bases, which are expected to be toxicologically equivalent to the free acid, except for the acute corrosive properties which are at least partly determined by the very low pH of strong solutions of the acid. One of the major uses of TCA is as a pre- and post-emergence herbicide for control of couch grass and wild oat grass. For this use it is principally handled as the sodium salt.

IARC (1995) concluded that TCA is not classifiable as to its carcinogenicity in humans and that there is limited evidence for carcinogenicity in animals (Group 3). However this evaluation was based on two earlier reports only: since that evaluation significant additional evidence has been reported.

Carcinogenicity data available:

Epidemiological studies

No data on long-term effects of human exposure to TCA were found in an earlier search by IARC (1995) or more recently by OEHHA. However, IARC (1995) noted that trichloroacetic acid is a metabolite of trichloroethylene and tetrachloroethylene which is detectable in the urine of exposed humans. There are epidemiological studies involving occupational and community exposure to these compounds. With regard to human health effects of trichloroacetic acid the evidence is inadequate (IARC, 1995).

Animal bioassays

A number of bioassays have been reported which indicate that TCA is an hepatocarcinogen in the mouse. The male is more sensitive than the female. In the rat, TCA acid is hepatotoxic but not hepatocarcinogenic. The only studies considered by IARC (1995) were those reported by Herren-Freund *et al*. (1987) and Bull *et al*. (1990).

1. Mouse long-term drinking water study (61 weeks): Herren-Freund *et al*., 1987. Male $B6C3F_1$ mice received water containing 0 or 5 g/L TCA neutralized with sodium hydroxide to a pH of 6.5-7.5 for 61 weeks. Treated mice had a statistically significant increase in hepatic adenomas (8/22 versus 2/22 in controls) and hepatocellular carcinomas (7/22 versus 0/22 in controls). IARC (1995) concluded that the increased incidence of hepatocellular adenomas and carcinomas was treatment related.

2. Mouse long-term drinking water studies (52 weeks or 37 weeks + 15 weeks observation): Bull *et al*., 1990. Several groups of $B6C3F_1$ mice received TCA in their drinking water for a significant portion of their lifespan. The groups were as follows: a group of 11 males received 1 g/L TCA for 52 weeks; a group of 24 males received 2 g/L TCA for 52 weeks; a group of 11 males received 2 g/L TCA for 37 weeks and then water alone until week 52; two groups of females (10/group) received 0 or 2.0 g/L TCA for 52 weeks. Two groups of 35 and 11 male control mice were kept for 52 weeks. Macroscopic examination of the livers and kidneys of male mice revealed hyperplastic or neoplastic changes in livers of 2/35 controls, 5/11 low-dose, 19/24 high-dose, 4/11 high-dose exposed for 37 weeks. Histological confirmation of a portion of the treated mice revealed hepatocellular carcinomas in all mice examined (2 low-dose, 4 high-dose and 3 high-dose exposed for 37 weeks). None of the 35 male controls examined histologically had hepatocellular carcinomas. No hyperplastic nodules or neoplastic lesions were seen in either group of female mice. IARC (1995) concluded that the

incidence of treatment-related liver tumors increased in male mice, but also that the study had a lack of detailed reporting of the results.

3. Mouse long-term drinking water studies: DeAngelo and Daniel, 1990; DeAngelo, 1991.

   b) Male $B6C3F_1$ mice received water containing 0.0, 0.05, 0.5 or 5 g/L (0, 8, 71 and 595 mg/kg bw/day mean daily dose) TCA for 60 weeks. The actual number of mice/group was not specified. Prevalence of hepatocellular tumors (adenomas and carcinomas) was increased in the groups of male mice receiving 0.5 and 5 g/L TCA (37.9% and 55.2% respectively, compared to 13.3% in the control group). Tumor prevalence was not significantly increased in the group receiving 0.05% TCA.

   c) In a second experiment, male $B6C3F_1$ mice received water containing 0.0 or 4.5 g/L (0 and 583 mg/kg bw/day mean daily dose) TCA for 94 weeks, which was considered to be a lifetime exposure. Prevalence of hepatocellular tumors was increased in the exposed group (86.7%, compared to 15% in the control group).

   d) Female $B6C3F_1$ mice received water containing 0.0, 0.5 or 4.5 g/L (0, 71 and 583 mg/kg bw/day mean daily dose) TCA for 104 weeks. Prevalence of hepatocellular tumors (adenomas and carcinomas) was increased in the group of female mice receiving 4.5 g/L TCA (60% respectively, compared to 7.7% in the control group). Tumor prevalence was not significantly increased in the groups receiving 0.5 g/L TCA.

   The authors concluded that TCA has carcinogenic activity in male and female $B6C3F_1$ mice.

1. Mouse long-term drinking water study (360 or 576 days): Pereira, 1996. Female $B6C3F_1$ mice received water containing 2.0, 6.67, or 20.0 mmol/L TCA for 360 or 576 days. A statistically significant increase in hepatocellular carcinomas occurred in mice at the high dose compared to controls following 360 days of administration (5/20 versus 0/40, 0/40 and 0/19 in control, low-, and mid-dose mice, respectively). At the end of 576 days of administration, there was a statistically significant increase in hepatocellular adenomas in high-dose mice compared to controls (7/18 versus 2/90, 4/53 and 3/27 in control, low- and mid-dose mice, respectively) and a statistically significant increase in hepatocellular carcinomas in high- and mid-dose mice compared to controls (5/18 and 5/27 versus 2/90 in controls and 0/53 at the low-dose). The authors concluded that TCA has carcinogenic activity in female $B6C3F_1$ mice.

2. Rat long-term drinking water study (104 weeks): DeAngelo and Daniel, 1992; DeAngelo, 1991. Male F344 male rats received water containing 0.0, 0.05, 0.5 or 5 g/L (0, 3.6, 36 and 378 mg/kg bw/day mean daily dose) TCA over a 104 week period. Tumor prevalence was 4.2%, 15% and 9.1% for 0.05, 0.5 and 5 g/L TCA respectively and compared to 4.4% in the control group (actual number of rats/group not specified). The authors concluded that TCA was not carcinogenic in male F344 rats.

Other relevant data

TCA has been observed to act as a tumor promoter in mice treated with an initiating dose of N-methyl-N-nitrosourea (Pereira and Phelps, 1996; Pereira *et al.*, 1997) and in rats treated with N-nitrosodiethylamine (Parnell *et al.*, 1988).

TCA has been tested in a number of assays for genotoxic activity; the results are negative apart from the induction of DNA strand breaks and chromosomal effects. TCA did not induce λ prophage in *Escherichia coli* and was not mutagenic to *Salmonella typhimurium* strains in the presence or absence of metabolic activation (DeMarini *et al*., 1994; Nestmann *et al*., 1980). TCA did not induce DNA strand breaks in mammalian cells *in vivo* in one study (Chang *et al*., 1992), but in two other studies, induced DNA strand breaks in the livers of mice and rats treated up to 4 hours previously with TCA (Nelson and Bull, 1988; Nelson *et al.*, 1989). Chromosomal aberrations were not induced in human lymphocytes exposed *in vitro* to TCA (Mackay *et al*., 1995) while chromosomal aberrations and micronuclei were induced in bone marrow cells of Swiss mice injected with TCA (Bhunya and Behera, 1987). No micronucleus induction was observed when TCA was injected at approximately 10-fold higher doses into C57BL/JfBL/Alpk mice (Mackay *et al*., 1995).

TCA, and various other compounds of which TCA is a metabolite, induce peroxisome proliferation in mouse liver (Elcombe *et al.*, 1985; Larson and Bull, 1992). It has been argued that this response is important in the mechanism of carcinogenesis by these compounds. It is also noted that the peroxisome proliferation response is much greater in rodent liver than in other tissues or other species, although the significance of these observations is unclear both with regard to TCA and to other peroxisome proliferation inducers.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over TCA due to evidence of carcinogenicity from multiple experiments in male and female mice by a likely route of exposure (i.e., drinking water). The evaluation by IARC (1995) found only limited evidence of carcinogenicity in animals. This may reflect the limited data with which they were dealing, which has since been significantly augmented, including the observation of carcinogenicity in female as well as male mice. On the other hand, the lack of carcinogenicity observed in the rat, together with the mainly negative findings of genotoxicity in short-term tests, serve to temper the level of concern. IARC (1995) also considered the possible role of peroxisome proliferation (which may be a rodent-specific response), and metabolic differences between mice, rats and humans.

There a **HIGH** level of **concern over the extent of exposure** to TCA due to its occurrence as a contaminant of drinking water disinfected by chlorination. This results in universal exposure of the population of California to low levels of TCA. Other occurrences and industrial or agricultural uses affect a much smaller number of people, but have the potential to result in high exposures to those particular individuals.

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CARCINOGENICITY DATA SUMMARY: BIS(2-CHLORO-1-METHYLETHYL)ETHER

Bis(2-chloro-1-methylethyl)ether (BCMEE; bis(β-chloroisopropyl)ether; DCIP; dichlorodiisopropyl ether; CAS No. 108-60-1) has been used as an extractant, a solvent for fats, waxes and greases, an ingredient in paint and varnish removers and in spotting and cleaning solutions, a component in textile processing, a nematocide (in Japan) (IARC, 1986), and as an intermediate in the manufacture of dyes, resins, and pharmaceuticals (NTP, 1982). There is no evidence that it is currently being used in any of these applications in the US (IARC, 1986). Production in the US was estimated at over 30 million pounds in 1975 (NTP, 1982), but it is no longer produced commercially in the US (IARC, 1986). Bis(2-chloro-1-methylethyl)ether may be formed as a by-product during the manufacture of propylene oxide and propylene glycol and, based on US propylene oxide production levels in 1983, IARC (1986) estimated that 4 million pounds of bis(2-chloro-1-methylethyl)ether may be formed and released into air or water each year in the US. However, one US manufacturer of propylene oxide stated that changes in the manufacturing process have eliminated the formation of bis(2-chloro-1-methylethyl)ether as a by-product (NTP, 1982). Bis(2-chloro-1-methylethyl)ether has been detected in effluents from industrial plants, in several rivers (e.g., the Ohio (0.5-5 μg/l), Kanawha, and Mississippi Rivers in the US and the Rhine and Scheldt Rivers in the Netherlands), and in tap water (0.8 μg/l) from the Ohio River (IARC, 1986).

IARC reviewed bis(2-chloro-1-methylethyl)ether in 1986 and 1987, and concluded that there are no data available on the carcinogenicity in humans and that there is limited evidence in animals (Group 3).

Carcinogenicity Data Available:

Epidemiological Studies

No studies of the long-term health effects of human exposure to bis(2-chloro-1-methylethyl)ether were identified in the published literature by IARC (1986) or in recent literature searches performed by OEHHA.

Animal bioassays

1. Mouse long-term gavage studies (104 weeks): NTP, 1982. Groups of 56 $B6C3F_1$ mice/sex/dose received 0, 100, or 200 mg/kg body weight/day technical grade bis(2-chloro-1-methylethyl)ether (containing 25.9-28.5% 2-chloro-1-methylethyl(2-chloro-*n*-propyl)ether and 2.1-2.6% bis(2-chloro-*n*-propyl)ether) in corn oil by gavage 5 days/week for 103 weeks. Animals were observed for an additional week. Two batches of test material were used containing 69.4% and 71.5% bis(2-chloro-1-methylethyl)ether, respectively. The first batch was administered from weeks 1 to 94, the second batch from weeks 95 to 103. There was a positive dose-response trend in the incidence of alveolar/bronchiolar adenomas in males (5/50; 13/50; 11/50; $P < 0.05$) and females (1/50; 4/50; 8/50; $P < 0.02$). The incidence in high-dose females was statistically significant ($P < 0.03$) when compared with control incidences. The incidence of alveolar/bronchiolar carcinomas and adenomas (combined) was statistically significant in the life table and incidental tumor trend tests (6/50; 15/50; 13/50, $P < 0.05$ for males; and 1/50; 4/50; 10/50, $P < 0.01$ for females) and pair-wise comparisons with controls were significant at the $P \leq 0.04$ level for low- and high-dose males and at the $P \leq 0.01$ level for high-dose females In males, statistically significant dose-related increases ($p < 0.01$) in the incidences of hepatocellular carcinomas (5/50; 13/50; 17/50) and hepatocellular adenomas and carcinomas (combined) (13/50; 23/50; 27/50) were observed. Small increases in the incidences of rare squamous cell papillomas and carcinomas of the stomach and forestomach of female mice were also observed. NTP (1982) concluded: "Under the conditions of this bioassay, bis(2-chloro-1-methylethyl)ether—containing 2-chloro-1-methylethyl(2-chloropropyl) ether—was carcinogenic for $B6C3F_1$ mice, causing increased incidences of alveolar/bronchiolar adenomas in males and females and hepatocellular carcinomas in males. In addition, the occurrence of a low incidence of squamous cell papillomas or carcinomas in the stomach or forestomach of females (a rare tumor in $B6C3F_1$ mice) was probably associated with the administration of bis(2-chloro-1-methylethyl)ether."

2. Mouse long-term feeding studies (104 weeks): Mitsumori *et al*., 1979. Groups of 56 SPF ICR mice/sex/dose were fed diets containing 0, 80, 400, 2000 or 10000 mg/kg bis(2-chloro-1-methylethyl)ether (purity 98.5%) for

104 weeks. Seven animals/sex/group were killed at weeks 13, 26, and 52 and six animals/sex/group at 78 weeks. The numbers of animals per group that survived to week 104 (ordered by increasing dose) were for males: 8, 5, 8, 5 and 6 and for females: 5, 9, 9, 7, and 1. No treatment-related tumors were reported for either sex, however, the IARC Working Group (1986) noted that the study was designed to test chronic toxicity and had limited sensitivity for detecting carcinogenicity.

3. Rat long-term gavage studies (104 weeks): NCI, 1979. Groups of 50 Fischer 344 rats/sex/dose received 0, 100, or 200 mg/kg body weight/day technical grade bis(2-chloro-1-methylethyl)ether (containing ~31% 2-chloro-1-methylethyl(2-chloro-*n*-propyl)ether and ~4% bis(2-chloro-*n*-propyl)ether) in corn oil by gavage 5 days/week for 103 weeks, and observed for an additional week. Three batches of test material were used, each containing ~65% bis(2-chloro-1-methylethyl)ether. Fifty untreated animals/sex were included as controls, in addition to the vehicle controls. No treatment-related tumors were observed in either sex.

Other relevant data

Bis(2-chloro-1-methylethyl)ether was mutagenic to *Salmonella typhimurium*, both with and without metabolic activation (IARC, 1986). It caused chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells (NTP, 1982). It was negative in a *Drosophila melanogaster* sex-linked recessive lethal mutation assay and in an *in vivo-in vitro* rat hepatocyte unscheduled DNA synthesis assay (IARC, 1986).

Bis(2-chloro-1-methylethyl)ether is a beta-haloether. Two structurally related chemicals which are listed as carcinogens under Proposition 65 are the structural analog bis(2-chloroethyl)ether, which induces liver cell tumors in male and female mice (NTP, 1982), and the alpha-haloether bis(chloromethyl)ether, which induces malignant tumors in the respiratory tract of mice, rats, and humans (NTP, 1982; Mitsumori *et al*., 1979).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** for bis(2-chloro-1-methylethyl)ether since tumors were observed at multiple sites (e.g., liver, lung) in both sexes of the mouse. The level of concern is reinforced by strong structural analogies with known carcinogens. Tumors induced by these structurally related carcinogens occur at sites in common with tumors observed in bis(2-chloro-1-methylethyl)ether treated (i.e., liver and lung). The level of concern is tempered somewhat by the presence of the impurities 2-chloro-1-methylethyl(2-chloro-*n*-propyl)ether and bis(2-chloro-*n*-propyl)ether in the test compound administered to the mice in these experiments. This is counterbalanced by the positive genetic toxicity results. IARC (1987) classified bis(2-chloro-1-methylethyl)ether as a group 3 carcinogen.

There is a **MEDIUM** level of **concern over the extent of exposure** to bis(2-chloro-1-methylethyl)ether since it is unclear whether it is currently used in the US, and to what extent, if any, it is currently formed as a by-product during the manufacture of propylene oxide and propylene glycol. It has been detected in river water and drinking water in the US and abroad. It is stable in aqueous media and nonbiodegradable in river water (NTP, 1982); exposure to the general population is expected to occur through the consumption of contaminated drinking water (HSDB, 1995). Exposure to the compound in occupational settings would be predicted to occur through inhalation of vapors and eye and skin contact (HSDB, 1995).

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CARCINOGENICITY DATA SUMMARY: 2-AMINOFLUORENE

2-Aminofluorene (CAS number 153-78-6; also known as 2-fluorenylamine and 2-fluorenamine) is a synthetic arylamine compound used as a research chemical and experimental carcinogen. 2-Aminofluorene has not been formally evaluated by IARC or by regulatory agencies, although it is listed by NIOSH as "carcinogenic by RTECS criteria" in the RTECS (1997) database.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to 2-aminofluorene were identified by OEHHA's search of the scientific literature.

Animal bioassays

The RTECS database (NIOSH, 1997) identified 5 studies that reported positive results in male and female rats or mice treated orally, on skin, or by implant. The studies are all a number of years old and have methodological inadequacies, particularly as regards the duration of the studies, size of the exposed groups, and limited reporting. Since the effect of these deficiencies is in most cases to diminish the power of the studies to detect carcinogenicity, it is noteworthy that 2-aminofluorene nonetheless shows a consistent and severe effect in these studies. A number of other early studies in rats, mice and guinea pigs are reported in PHS149 (US-DHEW, 1994 and earlier), by various routes such as oral, subcutaneous or intraperitoneal injection, and skin painting. These include some positive and some negative results but overall add little to the evidence due to their small size, limited design and reporting, and in the case of the injection studies, questions about the relevance of the route of administration used.

1. Rat chronic dietary studies (8 months + 2 months observation): Miller *et al*., 1955. Groups of male and female Holtzman rats received 1.62 mmols/kg (294 ppm) 2-aminofluorene in diet for 8 months (32 weeks). Control groups received plain diet: all animals received plain diet for a further 2 months, giving a total observation period of 10 months. In rats receiving control diet, the only tumors reported were mammary gland adenocarcinoma (1/20 at 10 months) among female rats and lung adenoma (1/18) among male rats. Exposed female rats developed mammary gland adenocarcinomas: 1/9 at 5 months, 4/9 at 8 months, and 7/9 at 10 months. In addition, 5/9 had ear duct tumors. Exposed male rats developed liver tumors (hepatomas, malignant cholangiomas; 9/9), ear duct tumors (sebaceous gland carcinomas or squamous cell carcinomas; 5/9), and small intestine adenocarcinomas (4/9) (all observed at 10 months). The increased incidences noted at 10 months in both male and female rats were statistically significant relative to controls ($P < 0.01$ by Fisher's exact test).

2. Rat chronic dietary studies (23 weeks + observation until death or significant morbidity): Morris *et al*., 1950. A group of 11 Minnesota rats (5 males and 6 females) received 50 ppm 2-aminofluorene in their diet (estimated total dose 537 mg per rat). Dosing was continued for 23 weeks after which observation continued, while the animals received control diet, until the animals were dead or moribund. One male rat developed a liver cholangioma. Among the 6 females, 2 cholangiomas, 5 mammary adenocarcinomas ($P < 0.05$ relative to control, Fisher's exact test), and 1 squamous cell carcinoma of the ear duct were observed. No tumors were observed among six rats (3 of each sex) receiving control diet.

3. Rat chronic skin studies (23 weeks + observation until death or significant morbidity): Morris *et al*., 1950. A mixed group of 11 rats (which included 10 Minnesota strain rats, 1 Wistar rat, six males and five females) received 2% 2-aminofluorene in acetone 3 times weekly (estimated total dose 53 mg per rat) by skin painting. Dosing was continued for 23 weeks after which observation continued until the animals were dead or moribund. Mammary carcinomas were observed in 4 of the 5 females. Among the six males, 1 cholangioma, 3 squamous cell carcinoma of the skin, and 1 pituitary adenoma were observed. Only one of the squamous cell carcinomas was near the site of application of the compound. No tumors were observed among six rats (3 of each sex) painted with acetone only. (Total tumor incidence is significantly increased [$P < 0.01$], but due to the small size of the groups the individual results are not statistically significant. However, the high proportional incidence of mammary carcinomas in females and the consistency with the result in the feeding study suggest that that observation at least may be biologically significant.)

4. Mouse chronic (52 week) skin studies: Bielschowsky *et al*., 1960. Carcinogenicity of topically applied 2-aminofluorene was compared in two groups of mice carrying a gene for pituitary dwarfism: one group was homozygous (and therefore phenotypically dwarf) while the other was heterozygous (and therefore of normal size). Mice received 90 treatments with 4% 2-aminofluorene in acetone by skin painting. Dwarfs (average body weight 7 - 8.5 g) received approximately 135 mg total dose (17420 mg/kg body weight), whereas normal sized mice (average body weight 23-26 g) receiving a total of 270 mg (11020 mg/kg body weight). 39 dwarf mice of an initial 55 treated with the carcinogen survived for at least 29 weeks, the time of first observation of a tumor. Liver tumors were increased in both types of mouse by treatment with 2-aminofluorene. Incidences of hepatomas in normal sized mice were 13/20 in males and 19/21 females. No such tumors were seen in 14 untreated males or 14 untreated females. In the dwarf mice hepatomas were seen in 7/17 males and 6/22 females. Only one hepatoma was observed in 37 control dwarf mice (sexes combined), although two additional benign tumors (considered histologically different from those seen in exposed animals) were noted. The incidences of hepatomas were statistically different from controls in all four exposed groups ($P < 0.01$, Fisher's exact test). In the exposed groups 4/41 normal sized mice and 4/39 dwarf mice had bladder carcinoma. No such lesions were seen in the 28 normal controls, and only 1/37 among the dwarf controls. One carcinoma of the kidney (pelvis) and two benign papillomas of the gall bladder were observed among 41 exposed normal-sized mice: no other incidences of these tumors were reported. Incidence of tumors (including adenocarcinomas) of the intestinal tract on either side of the pylorus was increased (12/41) in exposed normal mice. Incidence of duodenal tumors in the corresponding controls was 4/28, but these were described as small benign papillomas. Benign papillomas at this site were found in the dwarf mice: (exposed 3/39, controls 1/37). Mammary carcinomas were observed in 8/41 exposed normal sized mice and 2/28 controls. No mammary tumors were observed in either exposed or control dwarf mice. Lung tumors were the most common spontaneous tumor among the normal sized mice (11/28), but were not seen in either control or exposed dwarf mice. Exposed normal mice also developed lung tumors (5/41), but the different ages of the lung-tumor bearing groups (due to death from other tumors) make comparison of these incidences difficult.

5. Mouse implant study (40 weeks): Clayson, *et al*., 1958. Albino mice received 12.5% 2-aminofluorene in compressed cholesterol pellets implanted into the lumen of the bladder. Animals were observed for 40 weeks after the implantation. There was a survival rate of 100%. Among 38 mice exposed to 2-aminofluorene, 6 developed bladder carcinomas (3 invasive and 3 noninvasive) and 3 developed benign tumors of the bladder. In controls (implanted with a plain cholesterol pellet), 5/55 mice had bladder carcinomas, all of which were non-invasive, and one had a benign bladder tumor. The result for invasive tumors only is close to statistical significance ($P = 0.06$, Fisher's exact test), and might be considered biologically significant in relation to the positive observations in other studies.

Other relevant data

2-Aminofluorene has been used as a prototype compound in research on arylamine carcinogenesis: it has structural analogies with a number of well-known carcinogens including compounds identified as human carcinogens. It forms DNA and hemoglobin adducts.

Most systems that provide adequate metabolic activation indicate that 2-aminofluorene is genotoxic (Heflich and Neft, 1994). It is mutagenic in *Salmonella typhimurium* after metabolic activation (Duverger-van Bogaert *et al.*, 1995). According to data cited in RTECS (1997), it is active in the Syrian Hamster Embryo cell clonal assay, cell transformation in rat embryo cells, host mediated assay, *E. coli*, Ames test, sperm morphology in mice, and sister chromatid exchange (SCE) in a nonhuman system *in vitro* (dose-response). Negative results were obtained for *E. coli* without S9 and unscheduled DNA synthesis in human fibroblasts *in vitro*. Results were inconclusive for mammalian micronuclei and SCE *in vivo* (nonhuman) assays. 2-aminofluorene forms DNA adducts in various types of test system (Heflich and Neft, 1994).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over 2-aminofluorene since evidence of carcinogenicity has been observed at various sites in separate studies (using both skin and oral exposures), in both sexes of the rat and mouse.

This is reinforced by extensive evidence of genetic toxicity and by structural analogy with compounds known to be carcinogenic in animals and humans and listed under Proposition 65 (*e.g.* 2-naphthylamine, 2-fluorenylacetamide).

There is a **LOW** level of **exposure concern** for 2-aminofluorene since this chemical is used as a model research compound in mutagenicity and carcinogenicity studies. The level of concern is diminished by the health and safety procedures commonly employed in modern research laboratories, which are designed to minimize or eliminate potential exposures to laboratory workers, including such protective handling procedures as the use of glove boxes, hoods, gloves, and masks. The chemical would be expected to biodegrade very slowly, but it is not likely to bioconcentrate. No evidence was identified to suggest that any significant quantity of this chemical is present in the environment, although related compounds (*e.g.* 2-nitrofluorene) may occur as combustion by-products and could in theory be converted to 2-aminofluorene under some circumstances.

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CARCINOGENICITY DATA SUMMARY: 4-AMINO-2-NITROPHENOL

4-Amino-2-nitrophenol (CAS No. 119-34-6) is used as a hair dye and is used to dye furs. It was evaluated by the International Agency for Research on Cancer (IARC, 1987) and classified in group 3. This chemical has not been evaluated by the US Environmental Protection Agency as a possible carcinogen.

Carcinogenicity Data available:

Epidemiological studies

In its evaluation of 4-amino-2-nitrophenol, IARC (1977) stated that "No case reports or epidemiological studies were available to the working group." No epidemiological studies of humans exposed to this chemical have been identified in scientific literature published after the IARC review.

Animal bioassays

1. Rat long-term oral study (NCI, 1978): Groups of 50 male and 50 female Fischer rats were given feed supplemented with 1,250 ppm or 2,500 ppm 4-amino-2-nitrophenol, and groups of 20 male and female rats were given feed without 4-amino-2-nitrophenol for 103 weeks. In males, the incidence of transitional cell carcinomas of the urinary bladder was 0/15, 0/46 and 11/39 in control, low-dose and high-dose groups, respectively. The incidence in the high-dose group is significantly increased above that in controls ($p=0.018$) and the trend is significantly dose related ($p<0.001$). In females, the incidence of transitional cell carcinomas of the urinary bladder was 0/15, 1/43 and 2/44 in control, low-dose and high-dose groups. While the increased incidences in dosed females are not statistically significant, it is noted in the report that transitional cell carcinomas of the urinary bladder are very rare in untreated rats.

2. Mouse long-term oral study (NCI, 1978): Groups of 50 male and 50 female $B6C3F_1$ mice were given feed supplemented with 1,250 ppm or 2,500 ppm 4-amino-2-nitrophenol, and groups of 20 male and female mice were given feed without 4-amino-2-nitrophenol for 103 weeks. No significant increases in tumor incidence were found in treated mice of either sex.

3. Mouse long-term dermal study (Stenback *et al*, 1977): Groups of 50 female Swiss mice were given 2 dermal applications per week of 0.02 ml acetone containing 5% or 10% 4-amino-2-nitrophenol starting at 7 weeks of age and continuing until death occurred. When compared to a group of 100 untreated female mice, there were no significant increases in tumor incidences in treated groups.

4. Mouse injection study (Maronpot *et al*., 1986): Groups of 10 male and 10 female strain A mice were given 3 i.p. injections per week of 125, 62.5 or 31.25 4-amino-2-nitrophenol (in 0.1 ml tricaprylin) for 8 weeks. After 24 weeks, the incidence of lung tumors in these groups was not significantly increased above the incidence in 60 male and 60 female mice given tricaprylin alone.

5. Mouse injection study (Maronpot *et al*., 1986): Groups of 30 male strain A/j mice were given 3 i.p. injections per week of 40, 100 or 200 mg/kg 4-amino-2-nitrophenol in 0.1 ml corn oil for 8 weeks. At 24 weeks, the incidence of lung tumors in treated groups was not significantly increased above that in a group of 30 male mice injected with corn oil alone.

Other relevant data

4-Amino-2-nitrophenol is mutagenic in the bacterium *Salmonella typhimurium* in the presence of metabolic activation and is mutagenic in mouse lymphocytes (RTECS, 1997). It is structurally related to nitro- and amino-substituted benzene compounds found to be carcinogenic.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over 4-amino-2-nitrophenol because it produced a high incidence of very rare tumors (transitional call carcinomas of the urinary bladder) in male rats. A low incidence of

the same type of tumors was found in female rats given 4-amino-2-nitrophenol; while this incidence was not statistically significant, it may be biologically significant. (These results were not reviewed by IARC.) This concern is strengthened by positive tests for genotoxicity and chemical structural analogies with known carcinogens.

There is a **LOW** level of **concern over the extent of exposure** because it appears not to be produced in the United States (HSDB, 1997). No information on imports of this chemical has been found.

References

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CARCINOGENICITY DATA SUMMARY: ACRONYCINE

Acronycine (CAS No. 7008-42-6), an alkaloid derived from the bark of the Australian scrub ash, has been investigated as an experimental anti-cancer drug.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Rat long-term intraperitoneal injection studies (51 or 52 weeks + an additional 28-30 week observation period): NCI, 1978. Groups of 35 Sprague-Dawley rats of each sex were administered acronycine at one of three doses, 3.75 mg/kg, 7.5 mg/kg, or 15 mg/kg body weight. Control groups of each sex consisted of 10 untreated rats and 10 rats injected with the vehicle composed of 0.05% polysorbate 80 in phosphate buffered saline. In male rats, the dose-related trend in the mid- and high-dose groups for the incidence of osteosarcoma at all sites was statistically significant (p=0.002) (vehicle controls 0/8, mid-dose 13/30, high-dose 12/18). Comparisons of the individual groups with respective control groups were also significant for the mid-dose (p=0.022) and high-dose (p=0.002) groups. In female rats, adenocarcinomas of the mammary gland were observed in 7 low-dose, 5 mid-dose, and 2 high-dose female rats, but not in any control females. The reverse dose relationship of the mammary gland tumors was probably due to the higher number of early deaths which occurred in the high-dose group. Sarcomas of the peritoneum and other related tumors (mesothelioma, malignant mesothelioma, and fibrosarcoma of the peritoneum or multiple organs) were observed in all three dosed groups of both male and female rats, but in none of the control groups. The dose-response trends were significant in both sexes (p=0.006 in males and p=0.002 in females). Incidence in the high-dose females was significantly higher (p=0.016) than that of vehicle controls. None of the incidences in the individual dosed groups of males were significant when compared with vehicle controls. However, since the tumors were observed in all dosed groups but did not occur in historical-control animals at the laboratory, they are considered to be related to the administration of the chemical. NCI concluded that acronycine was carcinogenic in rats, producing tumors of the mammary gland in females, osteosarcomas in males, and sarcomas and other related tumors of the peritoneum in both males and females.

2. Mice long-term intraperitoneal injection studies (25 to 92 weeks, depending on toxicity): NCI, 1978. Groups of $B6C3F_1$ mice of each sex were administered acronycine at one of four doses, 2 mg/kg, 6 mg/kg, 12.5 mg/kg or 25 mg/kg body weight. The low survival in all dosed groups except the low-dose animals precluded meaningful evaluation. Lymphomas occurred in low-dose groups of both sexes; however, the incidence of lymphoma in different control groups was highly variable and may be associated with the possibility of transfer of tumor cells or oncogenic viruses during the intraperitoneal injection of the test chemical. NCI concluded that the bioassay in $B6C3F_1$ mice was inadequate.

3. Mice intraperitoneal injection studies: Stoner *et al*., 1973. Three dose levels were tested, the maximally tolerated single dose (MTD) (2.6 g/kg mouse), and a 1:2 and 1:5 dilution of the MTD, with 10 male and female A/He mice per dose. Five injections were given per dose and the experiments were terminated 24 weeks after the first injection. Two series of controls were maintained during the experimental period. One consisted of untreated mice and the other controls received injections of water, tricaprylin, or steroid suspending vehicle. Lungs of treated and control animals were examined using the A mouse pulmonary tumor system developed by Andervont and Shimkin. Under this system, acronycine did not increase pulmonary tumor incidence at the dose levels used.

Other relevant data

Acronycine has been shown to inhibit RNA and DNA synthesis in cultured mammalian tumor cells. It inhibited the accumulation of extracellular uridine and thymidine as nucleotides in the intracellular precursor pool by possibly altering the transport of the nucleosides through the plasma membrane (Dunn *et al*., 1973).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over acronycine since administration through i.p. injection to rats increased incidences of tumors of the mammary gland in females and rare tumors such as osteosarcomas in males, sarcomas and other related tumors of the peritoneum in both males and females.

There is **NO IDENTIFIED concern over the extent of exposure**. Acronycine and its derivatives have been investigated for use as a chemotherapeutic drug but there is no data to indicate that acronycine has been approved as a drug in the US.

References

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CARCINOGENICITY DATA SUMMARY: 2-AMINO-5-(5-NITRO-2-FURYL)-1,3,4-OXADIAZOLE

2-Amino-5-(5-nitro-2-furyl)-1,3,4-oxadiazole (ANFO; CAS No. 3775-55-1) was synthesized as a potential antimicrobial compound in the 1970s. The two important chemical functions are the nitrofuryl group which is responsible for its antiinfective properties (IARC, 1991) and the oxadiazole group that enhances the binding of ANFO to its receptor (Street *et al*.,1990). ANFO has not undergone carcinogen classification by IARC, USEPA, or NIOSH. No information on production levels or exposed populations was found in a recent OEHHA search.

Carcinogenicity Data available:

Epidemiological studies

No human studies were found in a recent OEHHA search.

Animal bioassays

1. Rat feeding study (46 weeks + 20 weeks observation): Cohen *et al*., 1975. Weanling female Sprague-Dawley rats (n=33) were fed ANFO for 46 weeks and observed for an additional 20 weeks. The cumulative dose was 4 grams ANFO/rat, administered in decreasing amounts over the 46 week interval. Treatment related mammary tumors (fibroadenomas and adenocarcinomas) were observed. Tumor incidence was based on number of rats alive in each group at week-10, when the first mammary tumor was palpated, and autopsies were performed when the rats died or were killed. Mammary tumor incidences among the rats were: fibroadenomas, controls-2/24, treated - 9/33; adenocarcinomas, controls-0/24, treated-20/33. The total mammary tumor incidence among the ANFO-exposed rats, 29/33, was statistically significant ($p << 0.001$) when compared to the control rats. Whereas the mean number of mammary tumors per rat with tumors was 1 for the control animals, the number for the ANFO-fed rats with tumors was 3.9. In addition to mammary tumors, kidney transitional cell carcinomas (5/33, $p=0.03$) and stomach squamous cell papillomas (6/33, $p=0.01$) were observed.

Other relevant data

No information on genotoxicity, metabolism, or toxicokinetics was found in a recent OEHHA search.

A chemically related compound, 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (CAS No. 712685), was also found to induce mammary tumors in female rats (Cohen *et al*., 1975), and is listed on the California Proposition 65 list of chemicals known to the state to cause cancer.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** for 2-amino-5-(5-nitro-2-furyl)-1,3,4-oxadiazole, based on the induction of a high incidence of mammary tumors and significant findings at other sites. Total mammary tumors, a high percentage of which were adenocarcinomas, occurred in 88 percent of the exposed female rats. In addition, elevated incidences of rare transitional cell carcinomas of the kidney were observed in the exposed rats, as well as benign forestomach tumors. A chemically related compound, 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (CAS No. 712685), is listed on the California Proposition 65 list of chemicals known to the state to cause cancer.

There is **NO IDENTIFIED CONCERN over the extent of exposure** for 2-amino-5-(5-nitro-2-furyl)-1,3,4-oxadiazole, because it was used only as a research chemical and there is no current production or use.

References

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CARCINOGENICITY DATA SUMMARY: N-BUTYL-N-NITROSOUREA

N-Butyl-N-nitrosourea (also known as butylnitrosourea; CAS No. 869-01-2) has been used as a research chemical, including use in a number of studies of carcinogenesis mechanisms and structure-activity relationships, but is not known to occur naturally or to be manufactured on a commercial scale. N-Butyl-N-nitrosourea has not been reviewed by IARC, although a substantial number of other nitroso compounds, including structural relatives such as N-methyl-N-nitrosourea, N-ethyl-N-nitrosourea and N-nitrosodi-n-butylamine, have been evaluated and found to show sufficient evidence of carcinogenicity (IARC groups 2A or 2B) (IARC,1987).

Carcinogenicity data available:

Epidemiological studies

No reports of studies of the effects of long-term human exposure to N-butyl-N-nitrosourea were identified in a search of the scientific literature by OEHHA.

Animal bioassays

A large number of bioassays of varying quality have been reported: a search of PHS 149 (US-DHEW, 1994 and earlier) and CCRIS (Chemical Carcinogenesis Research Information System) revealed 37 with results considered positive by the authors, and 22 with non-positive results. Most of the non-positive results are probably due to insufficient study power or methodological deficiencies. Four of the more substantial reports which include positive study results are summarized below.

1. Mouse subcutaneous study (1 year): Fujii & Nomoto, 1983. Male and female CD-1 mice were given 333 mg/kg bw N-butyl-N-nitrosourea in a 1% gelatin solution by injection into the nape of the neck, once, within 24 hours of birth. Increased incidence of lung adenomas and adenocarcinomas [males 16/27 (59%); females 19/23 (83%), compared to males 2/18; females 0/18 in controls; $P < 0.01$ by Fisher's exact test for both results], and liver adenomas adenocarcinomas [males 9/27 (59%); females 5/23 (83%), compared to males 1/18; females 0/18 in controls; $P < 0.05$ for both results] were observed.

2. Rat gavage study (40 weeks): Lijinsky & Kovatch, 1989. Male and female F344 rats were given doses of 0 or 15 mg/ml N-butyl-N-nitrosourea in 0.2 ml ethyl acetate and corn oil twice/week for 40 weeks (total dose 1.7 mmoles). Increased incidences of squamous cell carcinomas of the forestomach (10/12 exposed, 0/12 controls) and lung tumors (bronchiolar/alveolar carcinoma and adenoma, 11/12 exposed, 0/12 controls) were observed in the males. Squamous cell carcinomas of the forestomach (11/12 exposed, 0/12 controls), and tumors of the lung (11/12 exposed, 1/12 controls), mammary gland (7/12 exposed, 0/12 controls), and uterus (9/12 exposed, 2/12 controls) were observed in the females. All these incidences in exposed male and female animals were significantly increased relative to controls; $P < 0.01$ by Fisher's exact test.

3. Rat drinking water study (30 weeks). Takano *et al.*, 1990. Female Sprague-Dawley rats were given doses of 400 ppm butylnitrosourea (BNU) in their drinking water, ad libitum for the duration of the experiment (30 weeks) in an attempt to analyze stages of leukemogenesis. Of 20 rats, leukemia was observed in 16/20 rats: acute myeloblastic leukemia was observed in 13 (86%), acute myelomonocytic leukemia was observed in 2 (13%), and one rat (6%) was observed to have erythroleukemia.

4. Rat drinking water study (50 weeks). Takeuchi *et al.*, 1984. Male and female F344/DuCrj rats were administered N-butyl-N-nitrosourea at a concentration of 400 ppm in their drinking water. By the 50th week, there was an incidence of upper-digestive-tract tumors of 35/39 (90%) in males and 34/39 (87%) in females. Tumors of the esophagus and forestomach were the most frequently occurring. In sixteen (41%) of the female rats, vaginal tumors were induced.

Other relevant data

N-butyl-N-nitrosourea is positive in several genotoxicity assays, including a host-mediated assay, *Saccharomyces cerevisiae* gene conversion, and cytogenetics and SCE assays *in vitro*. N-butyl-N-nitrosourea induced mutations in

Chinese hamster V79 cells, and chromosomal aberrations in bone marrow cells of Long-Evans, Wistar, & Sprague-Dawley rats. It was however negative in a rodent dominant lethal test, and in an assay *in vitro* for unscheduled DNA synthesis in human fibroblasts. N-butyl-N-nitrosourea is a direct-acting alkylating agent, and induces a similar pattern of tumors to those seen with other carcinogenic nitrosoalkylureas such as N-ethyl- and N-methyl-N-nitrosourea.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over N-butyl-N-nitrosourea since tumors have been reported at multiple sites in both sexes of rats and mice. The concern is reinforced by its positive activity in numerous genotoxicity tests *in vitro*, and by the structural similarity to other carcinogenic and genotoxic N-nitroso compounds.

There is **NO IDENTIFIED CONCERN over the extent of exposure** to N-butyl-N-nitrosourea. As reported by SRI in 1977 and 1979, and confirmed by Lijinsky (1996), this research chemical is not known to occur naturally; nor is it produced commercially in, or imported into, the U.S.

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CARCINOGENICITY DATA SUMMARY: 2,5-DIMETHOXY-4-AMINOSTILBENE

2,5-Dimethoxy-4-aminostilbene (CAS No. 23435-31-6) has been proposed as an intermediate for chemical synthesis (Weisburger *et al.*, 1978) and has no known current use. The International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency have not evaluated this compound as a possible carcinogen.

Carcinogenicity Data available:

Epidemiological studies

No studies of cancer in human populations exposed to 2,5-dimethoxy-4'-aminostilbene have been identified in the scientific literature.

Animal bioassays

1. Rat long-term oral study (Weisburger *et al.*, 1978): Groups of 25 male Charles River CD rats were given feed containing 0, 125 or 250 ppm 2,5-dimethoxy-4'-aminostilbene for 3 months. During the next 15 months, these groups were given feed containing 0, 62.5 or 125 ppm. After 18 months of treatment, all rats were fed a diet not containing 2,5-dimethoxy-4'-aminostilbene until all surviving animals were killed at 24 months and examined for tumors. Four additional control groups of male rats were observed concurrently in the testing of four other chemicals. Therefore, the matched control group was combined with the other concurrent control group to form the pooled control group. In the high-dose, low-dose, matched control and pooled control group, the incidence of tumors of the ear duct was, respectively, 8/24 (p=0.001), 8/23 (p<0.001), 0/16 and 1/111; the incidence of skin tumors was 5/24 (p=0.06), 4/23 (p=0.11), 0/16 and 0/111; the incidence of stomach tumors was 8/24 (p=0.001), 6/23 (p=0.03), 0/16 and 2/111; and the incidence of tumors of the small intestine was 6/24 (p=0.04), 3/23 (p=0.19), 0/16 and 0/111. The p-values were calculated using Fisher's exact test to compare the incidence with that in the matched control group. When the incidences are compared with the incidence in pooled controls, the value of p for tumors of the small intestine in the low-dose group is 0.005, and all other p values are less than 0.001 (highly significant). The number of malignant or benign tumors at these sites is not stated in the report. However, it is stated that 2,5-dimethoxy-4'-aminostilbene "induced numerous carcinomas of the ear duct . . . and squamous papillomas and carcinomas of the skin." It is also stated that "tumors appearing in the treated rats which were not seen in the controls included squamous neoplasms in the forestomach and adenocarcinomas and sarcomas of the small intestine."

2. Mouse long-term oral study (Weisburger *et al.*, 1978): Groups of 25 male and female albino CD-1 mice were given feed supplemented with 0, 2,000 or 4,000 ppm 2,5-dimethoxy-4'-aminostilbene for 18 months and were then fed the control diet for 3 months. All surviving animals were killed 21 months after the start of the experiment and examined for tumors. No significant increases in tumor incidence were found in dosed females. In males, the incidence of lung tumors at the low dose, 11/17, was significantly increased (p=0.002) above the incidence 24/99 in pooled controls and marginally increased (p=0.049) above the incidence 4/14 in matched controls. The incidence of lung tumors in the high-dose group, 7/20, was not significantly increased above the incidence in controls. There is no information on the number of benign and malignant lung tumors found in the groups of mice. However, it is stated in the report that "male mice, especially those receiving the lower dose, showed a significant increase in bronchiolar/alveolar adenomas and carcinomas of the lung." No increased incidences of tumors were reported in female mice.

Other relevant data

No studies of genotoxicity of 2,5-dimethoxy-4'-aminostilbene were found in the scientific literature. This compound is structurally similar to diethylstilbesterol and to tamoxifen, both of which are human carcinogens.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over 2,5-dimethoxy-4'-aminostilbene because it produced tumors of the ear duct, skin, small intestine and forestomach in male CD rats. Carcinomas of the ear duct and small

intestine are rare in untreated rats. The concern is strengthened by a significant increase in tumors of the lung in CD-1 male mice at the low dose, but not at the high dose. Chemical structural analogies with known carcinogens reinforce the level of concern.

There is **NO IDENTIFIED CONCERN** over the extent of exposure to 2,5-dimethoxy-4'-aminostilbene because no information has been found to suggest that it has ever been used in the United States.

Reference

Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen CG, Chu KC (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J Environ Pathol and Toxicol*, **2**:325-356.

CARCINOGENICITY DATA SUMMARY: N-ETHYL-N-FORMYLHYDRAZINE

N-Ethyl-N-formylhydrazine (CAS number 74920-78-8, also known as 1-ethylhydrazinecarboxaldehyde, EFH) is a synthetic chemical which has been produced in the laboratory as a structural homologue of N-methyl-N-formylhydrazine. EFH has not been reviewed by IARC.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to N-ethyl-N-formylhydrazine are available.

Animal bioassays

1. Mouse lifetime drinking water studies: Toth & Nagel, 1980. Groups of male (n = 50) and female (n = 50) Swiss albino mice were given EFH as a 0.02% solution in drinking water (maximum tolerated concentration as determined in a prechronic study) for the lifespan. The average daily intake of EFH was 2.26 mg for each male and 1.26 mg for each female. Untreated control groups consisted of 100 male or 100 female mice. The treatment substantially shortened the survival in both sexes when compared to that in the controls. Male mice developed significantly increased incidences of tumors in the lung (adenomas and adenocarcinomas), blood vessels (angiomas and angiosarcomas; mostly angiosarcomas, mainly located in the liver), liver (mostly benign hepatomas; 2 mice had hepatocellular carcinomas), gall bladder (adenomas and adenocarcinomas), and preputial gland (squamous cell papillomas and carcinomas). The incidences for male mice are as follows: lung: control 22/100, treated 39/50 ($P << 0.001$); blood vessels: control 5/100, treated 32/50 ($P << 0.001$); liver: control 2/100, treated 13/50 ($P << 0.001$); gall bladder: control 0/100, treated 4/50 ($P < 0.02$); and preputial gland: control 0/100, treated 5/50 ($P < 0.006$). Female mice developed significantly increased incidences of tumors in the lung (adenomas and adenocarcinomas) and blood vessels (angiomas and angiosarcomas; mostly angiosarcomas, mainly located in the liver) [lung: control 15/100, treated 49/50 ($P << 0.001$); blood vessels: control 8/100, treated 47/50 ($P < 0.001$)].

Other relevant data

Toth & Nagel (1980) investigated the activity of various substituted hydrazines in order to study the correlation between chemical structure and tumor development at specific organ sites. EFH was synthesized as a structural homologue of N-methyl-N-formylhydrazine, the most potent of several naturally occurring carcinogenic hydrazine compounds found in the false morel mushroom, *Gyromitra esculenta*. This is a wild species widely distributed in North America, including California, and is regarded as edible in spite of the known toxicity of the raw mushroom. Earlier studies by Toth & Nagel (1978, 1979) showed that N-methyl-N-formylhydrazine induced lung, liver, blood vessel, bile duct, and gall bladder tumors in Swiss mice. All but bile duct tumors were also induced by EFH. These data indicate that the formyl group appears to enhance the carcinogenic potency of alkyl hydrazines.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over EFH, based on the induction of tumors at multiple sites in male and female Swiss mice. Although only one species was tested, uncommon tumors were observed, and structure-activity studies by Toth & Nagel (1978, 1979) provide additional evidence in support of the carcinogenic potential of EFH.

There is **NO IDENTIFIED CONCERN over the extent of exposure** to EFH. EFH does not occur in nature, and the only known use is as a laboratory research chemical.

References

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CARCINOGENICITY DATA SUMMARY: CHLORTHAL-DIMETHYL (DCPA)

Chlorthal-dimethyl (2,3,5,6-tetrachloro-1,4-benzenedicarboxylic acid, dimethyl ester; chlorthal; dimethyl tetrachloroterephthalate; dacthal; DCPA; Dacthalor; CAS No. 1861-32-1) is a pre-emergence herbicide used for a variety of crops including food crops (particularly brassicas), cotton, annual grasses and ornamentals in the control of annual weeds. Annual release in California has been estimated at approximately 850,000 pounds. DCPA has been detected in market fruits and vegetables and in some groundwater samples. Sampling of water in the California aqueduct system under the State Water Project has shown detectable concentrations of DCPA frequently in the range of 0.04-0.07 µg/l (sampling in March, 1996; CDWR, 1997). Intake estimates ranging from 0.9-2.4 ng/kg_{bw}-day have been made for individuals ranging in age from 6 months to 65 years (HSDB, 1994; citing Gunderson, 1988). Technical grade DCPA may contain hexachlorobenzene and small amounts of dioxin (2,3,78-TCDD) as impurities (Extoxnet, 1997; citing Walker and Lawrence, 1992). US EPA has classified DCPA as a Group C carcinogen (US EPA, 1996).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to DCPA were found in a recent literature search by OEHHA.

Animal bioassays

1. Rat long-term feeding studies (1 or 2 years): unpublished studies by Ricera, Inc. summarized by CDPR, 1987. DCPA was administered in feed to Sprague-Dawley rats for 1 year (10/sex/dose) or 2 years (60/sex/dose) at concentrations of 0, 1, 10, 50, 500, or 1000 mg/kg-day. The compound was reported to be 97.7% pure with hexachlorobenzene contaminating at a level of 0.13%. An significant increase in combined hepatocellular adenomas and carcinomas and hepatocholangiocarcinomas was reported in females in the two highest dose groups (0/68, 0/69, 2/67, 1/68, 8/70, 11/69 with increasing dose; includes animals from the concomitant 1 year study; $p = 0.0036$ and $p < 0.0001$ for 500 and 1000 mg/kg-day, respectively, by Fisher's exact test). Hepatocellular carcinomas and hepatocholangioncarcinomas were also increased in the highest dose group (0/68, 0/69, 1/67, 0/68, 3/70, 5/69; $p = 0.03$). Significantly increased thyroid follicular cell adenomas were reported in males in the three highest dose groups (1/70, 1/69, 2/66, 8/64, 10/67, 7/68; $p = 0.012$, 0.0035, and 0.028 for the 50, 500, and 1000 mg/kg-day groups, respectively). In females, combined thyroid follicular cell adenomas and carcinomas combined were increased in the highest dose group (1/68, 1/69, 3/67, 4/68, 2/69, 7/69; $p = 0.033$). Thyroid follicular cell carcinomas alone were also marginally increased among female rats in the highest dose-group (0/68, 0/69, 1/67, 0/68, 1/69, 4/69; $p = 0.062$). Hepatic preneoplasia, defined here as eosinophilic foci, was reported in males in all but the lowest dose group (6/60, 10/59, 13/56, 21/54, 20/57, 17/59; $p < 0.05$).

2. Mouse long-term feeding studies (79 or 104 weeks): unpublished studies by Ricera, Inc. summarized by CDPR, 1987. DCPA was administered in feed to CD-1 mice for 27, 53, or 79 weeks (10/sex/dose) or 2 years (60/sex/dose) at concentrations of 0, 100, 1000, 3500, or 7500 ppm. Hepatocellular tumors (combined adenomas and carcinomas) were increased among female mice in the highest dose group (2/60, 1/60, 2/60, 4/60, 9/60 with increasing dose; $p = 0.027$ for the 7500 ppm group, by Fisher's exact test).

Other relevant data

DCPA has been demonstrated to be non-mutagenic in a number of tests including assays for mutation frequency and activation (*Salmonella* reverse mutation, L5178Y $TK^{+/-}$ forward mutation assays, CHO/HGPRT system), cytogenetic tests (chromosomal aberrations, SCE in CHO cells), DNA repair tests, and dominant lethal tests (reviewed by CDPR, 1987). A mouse micronucleus test with 99% pure DCPA showed an increase in the frequency of micronucleated polychromatic erythrocytes in male mice 48 hours following exposure by gavage (CDPR, 1987).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenicity concern** over DCPA because of evidence of increased tumor development in rats (liver and thyroid tumors in females and thyroid tumors in males) exposed in feed. Liver tumors were also increased among female mice similarly exposed. This level of concern is supported by a positive finding in the mouse micronucleus test. There is some concern, however, regarding contamination of the test chemical with known carcinogens, in this case hexachlorobenzene and possibly 2,3,7,8-TCDD.

There is a **HIGH** level of **concern over the extent of exposure** to DCPA. This herbicide is widely used in California both on commercial food crops and in residential control of annual weeds. Detectable levels have been found in food, and in groundwater samples from several regions of California. DCPA also has a relatively long half-life in soil and water.

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CARCINOGENICITY DATA SUMMARY: CYANAZINE

Cyanazine (2-chloro-4-(1-cyano-1-methylethylamino)-6-ethylamino-*s*-traizine; Bladex®; Fortrol®; CAS No. 21725-46-2) was introduced in 1971 by Shell Chemical Co. It is a substituted symmetrical triazine (*s*-triazine) herbicide used for control of annual grasses and broadleaf weeds and is registered for use on corn, cotton, grain, sorghum, and wheat fallow (Hayes and Laws, 1991). In California, 647,334 pounds were used in 1995, primarily on corn, wheat, uncultivated agricultural areas, and rights-of-way (CDPR, 1996). An estimated 36 million pounds of this chemical are used annually in the US, more than 95% of which are used on corn (Hayes and Laws, 1991). Residues in groundwater have ranged from 0.1 to 1 part per billion (Hayes and Laws, 1991). Cyanazine is persistent in the environment, and its degradation products (including metabolites from biological processes) are persistent and likely to be biologically active (US EPA, 1994b).

The US EPA has classified cyanazine as a Group C possible human carcinogen based on limited evidence from animal studies and inadequate data in humans (US EPA, 1994a). In November, 1994 US EPA initiated a Special Review of cyanazine, along with atrazine and simazine, based on the Agency's concern that long term exposure to these pesticides in food and drinking water may pose a risk of cancer. In 1995 the US EPA's Special Review of cyanazine was suspended as the result of an agreement reached with Dupont Agricultural Products (the current manufacturer) to phase out all cyanazine containing products effective December 31, 1999 (US EPA, 1995). At this time all sales and distribution by Dupont will be prohibited; existing stocks of the chemical may be used until Dec. 31, 2002. The International Agency for Research on Cancer has not reviewed cyanazine.

Carcinogenicity data available:

Epidemiological studies

An abstract for a population-based case-control study conducted in Iowa and Minnesota among 622 male farmers, aged 30 years and over, reports an association (relative risk=1.6) between cyanazine exposure and small cell lymphocytic lymphoma (Cantor *et al*., 1985). Another study among a similar population found that the odds ratio for developing leukemia from mixing, handling, or applying cyanazine was 0.9 (95% CI 0.5-1.6) (Morris Brown *et al*., 1990).

A population-based case-control study of white males aged 21 and over (172 cases, 516 age-matched controls) in Kansas found an association (odds ratio=2.5, 95% CI 1.2-5.4) between non-Hodgkin's lymphoma and use of triazine herbicides (specific herbicides not listed) (Hoar *et al*., 1986). An interview study among similar subjects concluded that there was no association (odds ratio=1.4, 95% CI 0.2-7.9) between colon cancer and use of triazine herbicides (specific herbicides not listed) (Hoar *et al*., 1985). Another population-based case-control study in northern Italy found an elevated risk for ovarian cancer in women considered to have been exposed to triazine herbicides (IARC, 1991).

Interpretation of each of these studies is limited by the fact that the study populations were subject to multiple chemical exposures; therefore the potential relative contribution of cyanazine to the observed increases in cancer risk can not be determined.

Animal bioassays

1. Rat long-term feeding studies (24 months): Bogdanffy, 1990, as reviewed in US EPA, 1994b and CDPR, 1991. Cyanazine was fed in the diet for 24 months at 0, 1, 5, 25, or 50 ppm to 62 Crl:CDxBR (Sprague-Dawley) rats/sex/group. A treatment related increase in mammary gland adenocarcinomas in females at 5, 25, and 50 ppm was reported. The incidences of malignant tumors (adenocarcinoma, carcinosarcoma, and fibrosarcoma combined) in each increasing dose group are 0/48, 6/43, 13/41, 18/47, 17/51. The increased incidences were primarily due to increases in adenocarcinomas. The authors reported that, based on historical incidences of malignant mammary neoplasms in eight two-year feeding studies using Crl:CDxBR rats, the observed tumor incidences are comparable to the upper range of historical values and could be reasonably explained by the random occurrence of spontaneous tumors. US EPA and CDPR, however, concluded that the observed increase in malignant mammary tumors was treatment-related (US EPA, 1994b; CDPR, 1991). The US EPA (1994b) noted that the incidences of malignant mammary gland tumors (adenocarcinoma and carcinosarcoma) were

statistically significant at the mid- and high-dose levels, with a statistically significant positive trend; the incidences were outside the historical control range of 10.1 to 22.7 percent (average 17.9 percent).

2. Mouse long-term feeding studies (24 months): unpublished, as reviewed in CDPR, 1991. Cyanazine was fed at 10, 25, 250, or 1,000 ppm for two years to 50 mice/sex/group. One hundred untreated mice of each sex served as controls. No tumorigenic effects were observed in either sex.

Other relevant data

When diluted in 10% dimethyl-sulfoxide, cyanazine had the highest genotoxic activity of 47 pesticides tested in a modified SOS microplate assay (Venkat *et al*., 1995). Cyanazine induced transformation of BALB/c-3T3 cells in the absence, but not the presence, of S9; it was cytotoxic to BALB/c-3T3 cells in the presence of S9 (Perocco *et al*., 1993). It was clastogenic in human lymphocyte cultures (Roloff *et al*., 1992) and mutagenic in mouse lymphoma L5178Y cells with and without rat liver activation, in a dose-responsive manner (CDPR, 1991). Cyanazine was not mutagenic in CHO-K1 cells (CDPR, 1991). Cyanazine was positive in a UDS assay (Jannasch and Sawin, 1986). Cyanazine was cytotoxic at $\geq$1 µM in primary rat hepatocytes and at 5,000 µg/plate in *Salmonella typhimurium* but no adverse effects were noted below 5,000 µg in *Salmonella typhimurium* (CDPR, 1991). It caused a significant increase in the rate of dominant lethal mutations in *Drosophila melanogaster* but this may have been due to a direct toxic effect to sperm (Murnik and Nash, 1977).

No toxicity was noted in human peripheral blood lymphocytes (HPBL) treated with cyanazine; the frequency of sister chromatid exchange was not significantly increased in HPBL cultured *in vitro* and treated with cyanazine (Hrelia *et al*., 1994). No increase in chromosome aberrations was reported in human lymphocytes (CDPR, 1991).

Several structurally-related triazine herbicides (e.g., atrazine, simazine) are reported to cause tumors in mammary glands of the female Sprague-Dawley rat (Mayhew *et al*., 1986; McCormick and Arthur, 1988). It has been hypothesized that breast cancer in humans may be linked to triazine exposure, based on the observation that triazines induce mammary tumors in mice (Davis *et al*., 1993).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenic concern** over cyanazine based on the observation of mammary tumors in female Sprague-Dawley rats in a single study. No carcinogenic effects were observed in male rats, or mice of either sex. The level of concern is reinforced by the observations of mutagenicity and genotoxicity in various short-term tests and by the observation that several structurally-related triazines also induce mammary tumors in female Sprague-Dawley rats. This level of concern is consistent with the suggestive findings of multiple population-based case-control studies which have observed elevated cancer risks associated with multiple chemical exposures, including exposure to cyanazine. The US EPA, which has classified cyanazine as a Group C possible human carcinogen (US EPA, 1994a), suspended a Special Review of the herbicide in 1995, as the result of an agreement reached with Dupont Agricultural Products (the current manufacturer) to phase out all cyanazine containing products effective December 31, 1999 (US EPA, 1995).

There is a **HIGH** level of **concern over the extent of exposure** since cyanazine is currently a highly utilized herbicide. Reported agricultural use in California has increased in recent years, from 348,644 pounds in 1992 (CDPR, 1993) to 532,752 pounds in 1994 (CDPR, 1995) and 647,334 pounds in 1995 (CDPR, 1996). Still, annual usage is expected to drop after the year 2000, when sales of this herbicide will be prohibited. Currently, occupational exposure to cyanazine occurs through dermal contact and inhalation of aerosols and dust, especially to workers applying the compound as an herbicide (HSDB, 1995). There is also the potential for exposure to contaminated ground and surface waters, as well as food (treated crops, residues in meat, milk, poultry, eggs) (US EPA, 1994b). In addition, this chemical may be present in rain and fog in areas of high use (US EPA, 1994b).

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CARCINOGENICITY DATA SUMMARY: DAPSONE

Dapsone (CAS No. 80-08-0) is a prescription drug used for the long-term treatment of leprosy (50-100 mg/day). It has also been used to treat dermatitis herpetiformis but this disease is not listed as an indication for dapsone in the United States. Dapsone has been used to treat coccidiosis in cattle. It does not appear to be produced in the United States; imports in 1979 were 2,860 kg (HSDB, 1997). From the recommended dosage of 50-100 mg/day, this quantity of dapsone would be sufficient to treat approximately 100,000 leprosy patients. Dapsone was evaluated by the International Agency for Research on Cancer (IARC, 1980) and classified in group 3. Dapsone has not been evaluated by the US Environmental Protection Agency as a possible carcinogen.

Carcinogenicity Data available:

Epidemiological studies

In its evaluation of dapsone, IARC (1980) stated that "Several cases of cancer have been reported in patients with dermatitis herpetiformis treated with dapsone. There was no evidence of an increased rate of cancer in patients with leprosy, many of whom would also have been treated with the drug. "

Animal bioassays

1. Rat long-term oral study: NCI, 1977. Groups of 35 male and 35 female Fischer rats were given feed supplemented with 600 ppm or 1,200 ppm dapsone for 78 weeks, and groups of 15 male and female rats were given feed without dapsone until all surviving rats were killed and examined for tumors at 104-108 weeks. In males, the incidence of fibrosarcomas or sarcomas of the spleen was 0/14, 0/34 and 6/32 in matched control, low-dose and high-dose groups, respectively, and the incidence of fibrosarcomas or sarcomas of the peritoneum was 0/14, 5/35 and 6/33 in matched control, low-dose and high-dose groups. No fibrosarcomas or sarcomas of the spleen or peritoneum were found in 43 controls from several untreated control groups (pooled controls) observed at the time of the bioassay. The increases in the incidence of fibrosarcomas or sarcomas in the spleen and in the peritoneum in high-dose males are statistically significant (p=0.005) when compared to the incidence in pooled controls. No significant increases in tumor incidence were found in treated females.

2. Rat long term oral study: Griciute and Tomatis, 1980. Groups of 76 male and 72 female BDIV rats were given 100 mg/kg dapsone suspended in water five times per week by intragastric intubation and control groups of 53 males and 53 females were given olive oil alone by intubation five days per week for 104 weeks. The mothers of the treated rats were given by intubation 100 mg/kg dapsone during the last two days of gestation and for 5 days per week during lactation. In males, the incidence of fibrosarcomas or angiosarcomas of the spleen was 4/44 in the dosed group compared with 0/49 in the control group. This increase in dosed rats is marginally significant (p=0.046). The incidence of thyroid C-cell carcinomas was 8/44 in dosed male rats compared to 2/49 in controls. This increase in treated rats is significant (p=0.03). In female rats, the incidence of thyroid C-cell carcinomas was 13/63 in treated rats compared to 3/49 in controls. This increase in treated rats is significant (p=0.03).

3. Rat oral study: Griswold *et al*., 1966. A group of 20 female Sprague-Dawley rats received a single intragastric dose of 100 mg dapsone in sesame oil. Surviving rats were killed 6 months later and examined for tumors. No tumors were found. However, this result is of limited significance because the duration of the study is short, the total dose is relatively low and the number of animals tested is small.

4. Rat oral study: Griswold *et al*., 1968. A group of 20 female Sprague-Dawley rats received a total dose of 300 mg dapsone in sesame oil administered over a 28-day period by intragastric intubation. Surviving rats were killed 9 months later and examined for tumors. No tumors were found. However, this result is of limited significance because the duration of the study is short, the total dose is relatively low and the number of animals tested is small.

5. Mouse long-term oral study: NCI, 1977. Groups of 35 male and 35 female B6C3F1 mice were given feed supplemented with 2,500 ppm or 5,000 ppm dapsone for 79 weeks, and groups of 15 male and female mice were

given feed without dapsone until all surviving mice were killed and examined for tumors at 104-108 weeks. No significant increases in tumor incidence in dosed animals were found.

6. Mouse injection study: Stoner *et al.*, 1973. Groups of 10 male and 10 female strain A mice were given 3 i.p. injections per week of approximately 100, 50 or 25 mg (in 0.1 ml carboxymethylcellulose-physiological saline solution) for 8 weeks. After 24 weeks, the incidence of lung tumors in these groups was not significantly increased above the incidence in 30 male and 30 female mice given injections of 0.1 ml of the vehicle alone. This result is of limited significance because the duration of the study is short.

Other relevant data

IARC (1980) stated that "Dapsone and its acetylated metabolites were not mutagenic to *Salmonella typhimurium."* Dapsone is structurally related to compounds found to be carcinogenic (e.g., aniline, *p*ara-chloroaniline).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenicity concern** over dapsone because it produced rare tumors (fibrosarcomas and sarcomas of the spleen and peritoneum) in male rats. In a second study it also produced sarcomas of the spleen in male rats as well as C-cell carcinomas of the thyroid in male and female rats. IARC (1980) stated that "in three different studies in rats, high doses of dapsone induced mesenchymal tumors of the spleen in males (and of the peritoneum in two studies)." However, IARC concludes that the evidence for carcinogenicity in experimental animals is "limited" without commenting on the apparent discrepancy between the weight of evidence and the classification. Structural chemical analogies with known carcinogens reinforce the level of concern

There is a **HIGH** level of **concern over the extent of exposure** because dapsone is used for the long-term treatment of leprosy. Thus, individuals with this disease represent a specific subpopulation chronically exposed to this chemical.

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CARCINOGENICTY DATA SUMMARY: 2,4-DICHLOROPHENOXYACETIC ACID AND ITS SALTS AND ESTERS

2,4-Dichlorophenoxyacetic acid (2,4-D; Hedonal®; Trinoxol®; CAS No. 94-75-7) and its salts and esters are some of the most commonly used herbicides in the US. 2,4-D and its salts and esters are also used as plant-growth regulators. Between 52-67 million pounds were estimated to be used in the US in 1990. These herbicides are used on a variety of crops, including corn, wheat, barley, oats, sugar cane, rice, citrus, stone fruits, grapes, nuts, asparagus, and on pastures, rights-of-way, for landscape maintenance, and forest management. In 1995, 25,003 pounds of 2,4-D and 669,932 pounds of various salts and esters of 2,4-D were used in California (CDPR, 1996). Workers may on average absorb about 0.1 mg 2,4-D/kg body weight per day (HSDB, 1995). 2,4-D and its salts and esters have been detected in drinking water and foods. The general population is estimated to be exposed to 0.3-2 µg 2,4-D and its salts and esters/kg body weight/day (HSDB, 1995). In one report, 2,4-D was detected at a maximum concentration of 9 ppb in the urine of 39 out of 197 potentially exposed children (HSDB, 1995). It is broken down by soil microorganisms and does not accumulate in soil as a result of normal agricultural use (IARC, 1986).

US technical grade 2,4-D is available as the free acid (98% purity) and as various salts and esters (IARC, 1986). In the past, hexachlorodibenzo-*p*-dioxin has been found to contaminate some samples of 2,4-D, however, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has not been identified as a contaminant.

2,4-D and its esters were reviewed by IARC in 1977; chlorophenoxy herbicides, including 2,4-D, were reviewed by IARC in 1986 and 1987. IARC (1987) classified chlorophenoxy herbicides, including 2,4-D, as Group 2B substances, based on limited evidence in humans and inadequate evidence (for 2,4-D) in animals. The dog epidemiological-like study (Hayes *et al*., 1991), several recent human epidemiological (Hoar *et al*., 1986; Woods *et al*., 1987; Pearce *et al*., 1987; Persson *et al*., 1989; Zahm *et al*., 1990; Zahm and Blair, 1992; Cantor *et al*., 1992, Smith *et al*., 1992; Johnson 1990; Bond *et al*., 1988; Wikland *et al*., 1986; Wikland *et al*., 1989; Wigle, 1990; IARC, 1990; as cited in US EPA, 1994) and several animal bioassays (Charles *et al*., 1996, Blakley *et al*., 1992, and numerous unpublished studies reviewed by CDPR, 1993) were not included in IARC's review.

A 1994 review (US EPA, 1994) by a joint committee of the US EPA Science Advisory Board (SAB) and Science Advisory Panel (SAP) concluded that the evidence for the carcinogenicity of 2,4-D was weakly suggestive. This review included all the above listed human epidemiology studies, except that of Asp *et al*. (1994). The US EPA does not currently list a classification for 2,4-D in its Integrated Risk Information System (IRIS).

The conclusions of IARC (1987) and US EPA (1994) regarding the human epidemiological studies are stated below, as well as a brief description of the study by Asp *et al*. (1994), the dog epidemiology study, and the animal bioassays.

Carcinogenicity Data available:

Epidemiological studies

Several human studies and one canine study have investigated the risk of cancer associated with 2,4-D. The interpretation of the human epidemiological evidence is complicated by exposure of most of the study groups to multiple herbicides.

Human Studies

IARC (1986) concluded that: "well-conducted case-control studies have provided the most information on the association between cancer and occupational exposure to chlorophenoxy herbicides. Statistically significant elevated odds ratios have been observed for cancers at some sites" [e.g., soft tissue sarcoma, non-Hodgkin's lymphoma, nasal and nasopharyngeal cancer], "but not consistently, in independent studies. The results of one cohort study on the incidence of soft-tissue sarcoma support the finding in case-control studies of an increased relative risk for these tumours. Other cohort studies have added little information. No consistent exposure-response relationship emerged from the different studies, and, in the studies that found an association, exposures were shorter than those usually associated with occupation-related cancers."

US EPA (1994) concluded in a review limited primarily to studies investigating the association between 2,4-D and NHL, "that the studies executed to date cannot distinguish whether observed risks reported are due to the use of 2,4-D or some other aspect of farming as an occupation."

Asp *et al*. (1994). An 18-year follow-up for mortality and cancer morbidity in a cohort of 1909 men occupationally exposed to 2,4-D and 2,4,5-T during the period 1955 through 1971 did not observe an increased risk of mortality or cancer morbidity for any tumor types in the study population. The authors noted the medium to low statistical power of the study.

Canine study

Hayes *et al*., 1991. A hospital-based case-control study of companion dogs (491 cases, 466 nontumor controls, 479 tumor controls) indicated that owners in households with dogs that developed malignant lymphoma applied 2,4-D herbicides to their lawn and/or employed commercial lawn care companies to treat their yard significantly more frequently than control owners (odds ratio=1.3, 95% CI 1.04-1.67). The risk of canine malignant lymphoma rose to a twofold excess with 4 or more yearly owner applications of 2,4-D. The authors noted that the findings are consistent with occupational studies in humans, reporting a modest association between agricultural exposure to 2,4-D and increased risk of non-Hodgkin's lymphoma, the histology and epidemiology of which are similar to those of canine malignant lymphoma.

Animal bioassays with 2,4-D

1. Mouse gavage/diet studies (gavage on days 7-28, then in diet until 78 weeks): Innes *et al*., 1969, as reviewed by IARC, 1977 and 1986 and Reuber, 1983. Groups of male and female (C57BL/6xC3H/anF)F_1 and (C57BL/6xAKR)F_1 mice (18/sex/dose) received 46.4 mg 2,4-D (90% purity)/kg body weight in 0.5% gelatin by gavage on days 7-28, after which 2,4-D was administered at 149 mg/kg or 323 mg/kg ((C57BL/6xAKR)F_1 mice only) in the diet though week 78. Groups of 79 male and 87 female (C57BL/6xC3H/anF)F_1 mice and 90 male and 82 female (C57BL/6xAKR)F_1 mice which were untreated or had received gelatin only served as controls. No treatment-related increases in tumor incidences were observed. The IARC working group noted the small numbers of animals used and the inadequate reporting. The less-than-lifetime duration of the study conducted in males also may not have been sufficient to detect a carcinogenic effect.

2. Mouse long-term feeding studies (2 years, males only 1 year): Charles *et al*., 1996 and as reviewed by CDPR, 1993. 2,4-D was fed to male and female B6C3F_1 mice at 0, 5, 150 and 300 mg/kg/day for two years. All groups of male mice were terminated at one year, however, due to high toxicity at the mid- and high-doses. CDPR noted an increase in hepatocellular adenomas in treated males. A non-statistically significant increase in hepatocellular adenomas was observed in females (5/50; 11/50; 8/50; 10/50). This increase was not considered treatment-related since there was no dose-response and since the incidences fell within the historical control range of the laboratory.

3. Mouse long-term feeding study (2 years): Charles *et al*., 1996. 2,4-D was fed to male B6C3F_1 mice at 0, 5, 62.5 and 125 mg/kg/day for two years. No treatment-related tumors were observed.

4. Mouse long-term drinking water study (1 year): Blakley *et al*., 1992. Male Swiss (CD-1) mice (50 animals/group) were administered 0, 10, 25, or 50 mg 2,4-D/kg-day in the drinking water for one year. No treatment related tumors were observed. The less-than-lifetime duration of this study may not have been sufficient to detect a carcinogenic effect, however.

5. Mouse long-term feeding studies: unpublished, as reviewed by CDPR, 1993. 2,4-D (97.5% purity) was fed to male and female B6C3F_1 BR mice at 0, 1, 15, and 45 mg/kg/day for 104 weeks. No tumors were reported, however, the CDPR noted the incomplete reporting, preventing an evaluation of the study results.

6. Rat long-term feeding studies (2 years): Hansen *et al.*, 1971, as reviewed by IARC, 1977 and 1986 and Reuber, 1983. Groups of 25 male and female Osborne-Mendel rats were fed for two years 0, 5, 25, 125, 625 or 1250 mg 2,4-D (96.7% pure) /kg of diet (i.e., ppm). The numbers of rats (male and female combined) with malignant tumors were 6 in controls and 8, 7, 7, 8 and 14 in the treated groups. Only the increased incidence of tumors

observed in the highest dosed group was reported as statistically significant (P<0.05) by the authors and IARC (1977); a trend test for total tumors was positive at the P < 0.05 level for both males and females (Hansen *et al*., 1971). The IARC (1977) working group noted the small numbers of animals used and the inadequate reporting. In a reanalysis of the histopathology, Reuber (1983) reported for males: an increase in the total incidence of carcinomas in the 25, 125, and 625 ppm dose groups (P < 0.05); an increase in the total incidence of sarcomas in the 125 and 1250 ppm dose groups (P < 0.05); an increase in the total incidence of carcinomas and sarcomas (combined) in the top 4 dose groups (P < 0.01 for all doses except 625 ppm, where P < 0.05; positive trend test, P < 0.05). The sarcomas observed in male rats were mostly lymphosarcomas (0/25; 2/25; 4/25; 5/25; 3/24; 6/23; P < 0.05 for 125, 1250 ppm dose groups; positive trend test P < 0.05). Reuber (1983) reported for females: an increase in the total incidence of carcinomas in the top 2 dose groups (P < 0.05); an increase in the total incidence of sarcomas in the top 4 dose groups (P < 0.05); an increase in the total incidence of carcinomas and sarcomas (combined) in the 125, 625 and 1250 ppm dose groups (P < 0.05). An increased incidence of lymphosarcomas was observed in all groups of dosed females (0/22; 5/20; 6/22; 6/23; 12/24; 6/25; P < 0.05). In addition, mammary gland tumors were increased in females. Reuber (1983) concluded that 2,4-D was carcinogenic in male and female rats.

7. Rat long-term feeding studies (104 weeks): unpublished, as reviewed by CDPR, 1993. 2,4-D, 97.5% purity, was fed to Fischer 344 rats, 60/sex/group, at 0, 1, 5, 15, or 45 mg/kg/day for 104 weeks. Astrocytomas were increased in males (number of animals with tumors were: 1, 0, 0, 2, and 6 for increasing dose groups). The study pathologist concluded that the increased incidence of astrocytomas in the high-dose group was incidental, since the appearance of these tumors did not fit the picture of commonly observed treatment-related astrocytomas (e.g., no evidence of decreased tumor latency, tumors only observed at the high dose, no preneoplastic lesions (gliosis) observed, all tumors were solitary, and none were more advanced in stage in the treated animals, as compared with the tumor observed in the control). The Fisher-Exact test pair-wise comparison was marginally significant for the high-dose group (P < 0.054) and the Cochran-Armitage trend test was highly significant (P<<0.001). The US EPA concluded that the MTD may not have been reached in the male rats.

8. Rat long-term feeding studies (2 years): Charles *et al*., 1996. 2,4-D, 96.4% purity, was fed to Fischer 344 rats, 60/sex/group, at 0, 5, 75, or 150 mg/kg/day for 104 weeks. Ten rats/sex/group were killed after one year. Only the high-dose and control animals were examined for astrocytomas and other brain neoplasms. The incidences of astrocytomas in control and high-dose males were 0 and 1, respectively; the incidences in control and high-dose females were 1 and 1, respectively. The authors concluded that no treatment-related tumors were observed in either sex, although a complete histological examination was not performed on animals.

Animal bioassays with salts and esters of 2,4-D

1. Mouse gavage/diet studies (gavage on days 7-28, then in diet until 78 weeks): Innes *et al*., 1969, as reviewed by IARC, 1977 and 1986 and Reuber, 1983. In experiments similar to those described above with 2,4-D, mice were given 2,4-D isopropyl, butyl or isooctyl esters (99%, 99%, and 97% pure) at doses of 46.4 mg/kg body weight from 7-28 days of age and there after in the diet at 11, 149 and 130 mg/kg, respectively, up to 78 weeks of age. No treatment-related increases in tumor incidences were reported by the authors or noted by IARC (1977) for any of the three substances tested. The IARC (1977) working group noted the small numbers of animals used and the inadequate reporting. In a reanalysis of the data, Reuber (1983) concluded that for 2,4-D isooctyl ester, no treatment-related tumors were observed, for 2,4-D isopropyl ester a marginally statistically significant increase in lung tumors was observed in male (C57BL/6 X C3H/Anf)F_1 mice (4/18 vs. 2/17 matched controls and 5/79 pooled controls; P< 0.06), and for 2,4-D butyl ester a non-statistically significant increase in reticulum cell sarcoma was observed in female (C57BL/6 X C3H/Anf)F_1 mice (3/18 vs. 4/87 pooled cases; P<0.1).

2. Mouse single subcutaneous (s.c.) injection studies: NTIS, 1968, as reviewed by IARC, 1977 and 1986 and Reuber, 1983. Groups of 18 male and female (C57BL/6xC3H/Anf) F_1 mice and (C57BL/6xAKR)F_1 mice were given single s.c. injections of 21.5 mg/kg body weight isooctyl ester of 2,4-D (97% pure) and observed for up to 78 weeks of age. Groups of 141 male and 154 female (C57BL/6xC3H/Anf) F_1 mice and 161 male and 157 female (C57BL/6xAKR)F_1 mice served as untreated or vehicle controls. Five out of 17 treated females vs.

5/157 control females of the second strain developed reticulum-cell sarcomas (P<0.01). The IARC (1977) working group noted the small numbers of animals used and the inadequate reporting.

3. Rat long-term feeding studies (27 months): Arkhipov and Koslova, 1974, as reviewed by IARC, 1977 and 1986 and Reuber, 1983. Groups of 120 male and 45 female random-bred rats were fed the amine salt of 2,4-D at a daily intake of one-tenth the LD_{50} (not specified). Two treated rats developed tumors (one mammary fibroadenoma, one hemangioma of the mesenterium) at 23 months, and one untreated rat had a mammary fibroadenoma at 27 months. The IARC (1977) working group noted the inadequate reporting.

Other relevant data

In general, 2,4-D has tested positive in some short-term *in vitro* tests for genetic toxicity, but negative when tested *in vivo*. As summarized by IARC (1987): 2,4-D did not induce dominant lethal mutations, micronuclei or sister chromatid exchanges (SCEs) in rodents treated *in vivo*. Pure 2,4-D did not induce chromosomal aberrations in human lymphocytes *in vitro*, but a commercial formulation did. 2,4-D induced SCEs and unscheduled DNA synthesis (UDS) in human cells *in vitro*. It did not induce SCEs but did induce mutation and inhibited intercellular communication in Chinese hamster cells *in vitro*. It induced somatic mutation in *Drosophila*, but conflicting results were obtained for induction of sex-linked recessive lethal mutations; it did not induce aneuploidy. 2,4-D caused chromosomal aberrations and was mutagenic in plants. It induced mutation, gene conversion and mitotic recombination in yeast. It was not mutagenic in bacteria or bacteriophage. The *n*-butyl and *iso*-octyl esters of 2,4-D were not mutagenic in bacteria.

In additional studies, 2,4-D, the dimethylamine salt, and the 2-ethylhexyl ester were negative in the Ames test, in the *in vivo* mouse micronucleus assay, and in the *in vitro* rat primary hepatocyte UDS assay (as reviewed by CDPR, 1993). 2,4-D was shown in one study to inhibit male mouse testicular DNA synthesis by 29% (as reviewed by CDPR, 1993).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenicity concern** for 2,4-D and its salts and esters, since some epidemiology studies in humans and the single study in dogs have reported modest associations between exposure to 2,4-D herbicides and increased risk for certain cancers (i.e., non-Hodgkin's lymphoma and soft tissue sarcoma in humans and malignant lymphoma in dogs). The observations that 2,4-D increased the incidence of lymphosarcomas, as well as the numbers of total tumors/animal in rats of both sexes in one series of experiments further supports this level of concern. Adding to the concern are various observations of tumors at other sites in rats and mice exposed to 2,4-D and related compounds (i.e., significantly increased astrocytomas in 2,4-D-treated male rats of another strain, elevated hepatocellular adenomas in 2,4-D-treated male and female mice, significantly increased reticulum-cell sarcomas in female mice following a single injection of 2,4-D isooctyl ester, elevated reticulum-cell sarcomas in 2,4-D isopropyl ester-treated female mice, and elevated lung tumors in 2,4-D isopropyl ester-treated male mice). The evidence of genotoxicity from some short-term tests reinforces the level of concern. IARC (1987) classified chlorophenoxy herbicides, including 2,4-D, as Group 2B substances.

There is a **HIGH** level of **concern over the extent of exposure** to 2,4-D and its salts and esters, since these compounds are widely used in California and the rest of the US as herbicides and plant growth regulators. The general population may be exposed through contact with treated lawns, with children representing a potentially highly exposed subpopulation by this means. The general population may also be exposed through consumption of contaminated food and drinking water. In addition, environmental exposures may occur as a result of spray drift from field applications. Occupational exposures may occur via the dermal and inhalation routes during the manufacture, formulation, and application of these compounds.

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CARCINOGENICITY DATA SUMMARY: DIMETHYL HYDROGEN PHOSPHITE

Dimethyl hydrogen phosphite (CAS No. 868-85-9) is used as a lubricant additive, as a fire retardant on nylon 6 fibers, cotton and cellulosic textiles, and γ-irradiated polyethylene, as an adhesive, as a stabilizer in oil and plaster, and as an intermediate in the synthesis of certain pesticides (IARC, 1990). It was evaluated by the International Agency for Research on Cancer (IARC, 1990) and classified in group 3 based on limited evidence in experimental animals and no data in humans. This chemical has not been evaluated by the US Environmental Protection Agency as a possible carcinogen.

Carcinogenicity Data available:

Epidemiological studies

In its evaluation of dimethyl hydrogen phosphite, IARC (1990) stated that "No (human) data were available to the Working Group."

Animal bioassays

1. Rat long-term oral study (NTP, 1985): Groups of 50 male Fischer 344/N (F344/N) rats were given 0, 100 or 200 mg/kg dimethyl hydrogen phosphite in corn oil, and groups of 50 female F344/N rats were given 0, 50 or 100 mg/kg. Administration was by intragastric intubation on 5 days per week for 103 weeks starting at 7 weeks of age. At 111 weeks of age, all surviving rats were killed and examined for tumors. The incidence of squamous cell carcinomas of the lung was 0/50, 0/50 and 5/50 in control, low-dose and high-dose males, respectively. The increase in the incidence in the high-dose group is statistically significant (p-0.02) by Fisher's exact test. The trend in these incidences is significantly dose related (p=0.034, incidental tumor test for trend). The incidences of alveolar/bronchiolar carcinomas were 0/50, 1/50 and 20/50 in control, low-dose and high-dose males. The increase in the incidence in the high-dose group is highly statistically significant (p<0.001), and the trend in these incidences is highly significantly dose related (p<0.001, incidental tumor test for trend). The incidence of alveolar/bronchiolar carcinomas in females were 0/50, 1/49 and 3/50 in control, low-dose and high-dose groups. The increase in the incidence in treated females is not significant (p=0.12 for the high-dose group compared with controls), but the trend in these incidences is marginally dose related (p=0.047, incidental tumor test for trend). The incidence of forestomach papillomas or carcinomas combined in males were 0/50, 1/50 and 6/50 in control, low-dose and high-dose groups. The increase in the incidence in the high-dose group is significant (p=0.012). NTP concluded that "Under the conditions of these gavage studies, there was clear evidence of carcinogenicity in male F344/N rats receiving dimethyl hydrogen phosphite, as shown by increased incidences of alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and squamous cell carcinomas of the lung and of neoplasma of the forestomach. There was equivocal evidence of carcinogenicity in female F344/N rats receiving dimethyl hydrogen phosphite, as shown by marginally increased incidence of alveolar/bronchiolar carcinomas of the lung and of neoplasms of the forestomach.

2. Mouse long-term oral study (NTP, 1985): Groups of 50 male and 50 female $B6C3F_1$ mice were given 0, 100 or 200 mg/kg dimethyl hydrogen phosphite in corn oil. Administration was by intragastric intubation on 5 days per week for 103 weeks starting at 7 weeks of age. At 110-112 weeks of age, all surviving mice were killed and examined for tumors. Survival and weight gain were decreased in high-dose males but not in other groups. No significant increases in tumor incidence were found in treated animals. NTP concluded that "there was no evidence of carcinogenicity in male or female B6C3F1 mice receiving dimethyl hydrogen phosphite at 100 or 200 mg/kg for 103 weeks"

Other relevant data

Dimethyl hydrogen phosphite was not mutagenic in the bacterium *Salmonella typhimurium* in the presence or absence of metabolic activation and was not mutagenic in the *Drosophila melanogaster* recessive lethal mutation test (NTP, 1985). As reviewed by IARC (1990), it did produce mutations in L5178Y mouse lymphoma cells and produced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells in the presence and in the absence of exogenous metabolic activation. This chemical has some structural similarity to the carcinogens tris (2-chloroethyl) phosphate and tris (2,3-dibromopropyl) phosphate.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM-HIGH level of carcinogenicity** dimethyl hydrogen phosphite because it produced alveolar/bronchiolar carcinomas and squamous cell carcinomas of the lung and neoplasms of the forestomach in male F344/N rats. Squamous cell carcinomas of the lung are relatively uncommon in rats. The concern is strengthened by positive tests for genotoxicity in mammalian cells and structural similarity to known carcinogens.

There is a **HIGH** level of **concern over the extent of exposure** because it has multiple uses, including use as a fire retardant for cotton and cellulosic textiles and nylon materials. Approximately 3 million pounds are produced in the United States per year (NTP, 1985).

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CARCINOGENICITY DATA SUMMARY. MALONALDEHYDE AND ITS SALTS

Malonaldehyde (MA; malondialdehyde; CAS No. 24382-04-5) is a metabolic end-product of prostaglandin biosynthesis and lipid peroxidation (Metzler, 1977; IARC, 1985; Shimizu *et al*.. 1981; references in Kautenianen *et al*, 1993). MA in foodstuffs exists in a bound form whereas MA derived from lipid peroxidation exists unbound (Hartman, 1983). MA is used as a bifunctional reagent in protein and nucleic acid structure studies (Metzler, 1977; Piche *et al*, 1988; Chaudhary *et al*., 1994 and references therein). No known physiologic function is associated with MA. A contribution to background levels of MA in animals may be intestinal flora (Kautenianen *et al*., 1993). Commercial quantities of MA are not produced in the USA, except the small amounts associated with its use as a laboratory reagent (IARC, 1985). The numbers of workers occupationally exposed to MA are not available (NIOSH, 1991). To maintain stability of the chemical, the sodium salt of MA is used (NTP, 1988). MA was classified as a Group 3 (not classifiable) carcinogen, based on no data in humans and inadequate data in experimental animals (IARC, 1985; 1987), and received a low priority rating for future evaluation of naturally occurring chemicals (IARC, 1993). NIOSH recommends MA should be considered as a potential occupational carcinogen (NIOSH, 1991).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure were reported in a recent literature search by OEHHA or in an earlier review by IARC (1987).

Animal bioassays

1. Rat long-term gavage studies (2-years): NTP, 1988. Female and male rats (F344/N) (50/sex/group) were exposed to the sodium salt of MA in water by gavage for 2 years. The doses were 0, 50, and 100 mg/kg (vehicle control, low dose (LD), and high dose (HD), respectively). Decreased body weights and decreased survivals were observed among the HD rats. Increased incidences of thyroid gland adenomas were observed in HD males (7/50, p=0.007-life table test). Increased incidences of thyroid gland adenomas and carcinomas (combined) were observed in the HD males (13/50, p=0.007-life table test) and females (7/50, p=0.003-life table test; p=0.045-incidental tumor test). The respective incidences among the vehicle controls were 4/50-males and 2/50-females. Among LD males, increased incidences of pancreatic islet cell adenomas (9/50, p=0.006-life table test; p=0.002-incidental tumor test) and adenoma or carcinoma (9/50, p=0.006-life table test, p=0.009-incidental tumor test) were observed. NTP (1988) concluded there was clear evidence for carcinogenicity in female and male rats.

2. Mouse long-term gavage studies (2 years): NTP, 1988. Female and male mice ($B6C3F_1$) (50/sex/group) were exposed to the sodium salt of MA in water by gavage for 2 years. The doses were 0, 60, and 120 mg/kg (vehicle control, low dose (LD), and high dose (HD), respectively). No evidence of carcinogenicity was found, and NTP (1988) concluded there was no evidence for carcinogenicity in mice.

3. Mouse drinking water study (2 years): Bird *et al*., 1982. Swiss female mice were exposed to the sodium salt of MA in drinking water for up to 12 months. The doses were 0, 0.1, 1.0, or 10 mg /kg body weight-day. MA-related deaths occurred at the high dose (HD), and among the major causes of death were lung cancer and lymphoma. Histopathologic analysis revealed a MA dose-related increase in liver hyperplastic nodules, hemangiomas, and hepatomas, none of which were statistically significant at $p<0.05$). Responses for the individual lesions, however, were not significant ($p>0.05$).

Other relevant data

MA (sodium salt) was not mutagenic in *Salmonella typhimurium* (TA100, TA1535, TA1537, or TA98), with or without metabolic activation, and it did not induce sex-linked recessive lethal mutation in Drosophila (NTP, 1988). MA (sodium salt) was mutagenic towards mouse L5178Y lymphoma cells (Yau, 1979; NTP, 1988). MA induced sister chromatid exchanges but not chromosomal aberrations in Chinese hamster ovary cells (NTP, 1988). In Chinese hamster ovary cells, *in vitro* exposure to MA resulted in a dose-dependent increase in DNA damage in

which the apparent frequency of cross-link damage was greater than strand breaks (Marinari *et al*., 1984). MA, generated *in vivo* in humans through the ingestion/digestion of polyunsaturated- and monounsaturated fatty acids, forms adducts with DNA, such that the levels associated with a diet high in polyunsaturated acids are greater than those associated with a diet rich in monounsaturated fatty acids (Fang *et al*., 1996).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenicity concern** for malonaldehyde, based on the carcinogenic effects in male and female rats and in female mice. The level of concern in reinforced by observations that MA was mutagenic towards mouse L5178Y lymphoma cells and induced sister chromatid exchanges and dose-dependent increases in DNA damage in Chinese hamster ovary cells. The observation that diet affects the level of MA-DNA adducts in humans supports this level of concern.

There is a **HIGH** level of **exposure concern** for malonaldehyde. Although commercial quantities are apparently not manufactured in the USA, MA is present in food and its levels will increase under oxidative conditions.

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CARCINOGENICITY DATA SUMMARY: ZEARALENONE

Zearalenone ((S)-(-)-3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecin-1,7(8*H*)-dione; 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone; Compound F-2; fermentation estrogenic substance; FES; CAS No. 17924-92-4) is a natural mycotoxin produced by members of the *Fusarium* group, and also belongs to the category of phytoestrogens (IARC, 1983; IARC, 1993). In the United States, it has been detected in a variety of agricultural commodities, such as hay feed, corn, pig feed, sorghum, dairy rations, and barley (IARC, 1983). Concentrations in these commodities have been found to range from the limit of detection to 2900 mg/kg (IARC, 1983). However, the number of positive results in these studies usually represented a small proportion of the total number of samples analyzed. In addition, the presence of obvious mold and mycotoxins may down-grade crops from appropriate for human consumption to animal feed use, thereby protecting consumers from excessive direct intake of this chemical (Kuiper-Goodman, 1990). Zearalenone has been detected in the milk and meat of cows fed diets containing zearalenone, although there is some indication that transmission to milk is minimal (Scott and Lawrence, 1988; Fink-Gremmels, 1989; Prelusky *et al.*, 1990). Still, in studies on human food, levels were shown to be as high as 289 mg/kg (Pfohl-Leszkowicz *et al*., 1995). Zearalenone is also used as a chemical intermediate in the production of zeranol (α-zearalenol), a mixture of diastereoisomers of the alcohol derivative, and can be commercially produced by submerged fermentation of glucose (IARC, 1983). Zeranol, one of these isomers, is used as a veterinary anabolic agent, and has been evaluated as a potential treatment for postmenopausal syndrome (HSDB, 1994; IARC, 1983). Zearalenone itself has estrogenic properties. In the United States, only one company produces zearalenone, as an intermediate in the manufacture of other chemicals.

IARC concluded there was limited evidence of carcinogenicity in experimental animals, but human data were inadequate to draw conclusions, leaving zearalenone unclassifiable as to its carcinogenicity (Group 3) (IARC, 1983; IARC, 1993).

Carcinogenicity Data available:

Epidemiological studies

Levels of zearalenone and *Fusarium* contamination were evaluated in samples of corn from regions of both high and low risk for esophageal cancer in southern Africa (Marasas *et al.*, 1979; Marasas *et al.*, 1981; Marasas *et al.*, 1988; reviewed in IARC, 1993). The initial evaluation showed higher levels of contamination in the regions of higher risk, however, an extension of the study to include a third region with an intermediate rate of esophageal cancer failed to show a correlation of *F. graminearum* infection with the cancer rates. Quantitative information for evaluation of the data was not available. A third study identified 12 households in the high-risk region in which one or more adult occupants showed some degree of esophageal abnormality and 12 random households from the low-risk region (Marasas *et al.*, 1988). Ears of home-grown corn were sorted into groups intended for consumption in porridge (healthy ears) and for the brewing of beer (moldy ears) by the housewife of each household. The cancer risk was not found to be correlated with the occurrence of *F. graminearum* in the healthy corn, however it was inversely correlated with the occurrence of moldy corn. Analysis of some of the corn from this study showed significantly higher levels of zearalenone (and another mycotoxin nivalenol) in the moldy corn from the low cancer risk area relative to those in the high risk area (Sydenham *et al.*, 1990). The studies may be confounded by the presence of other mycotoxins.

Animal bioassays

1. Mouse long-term feeding studies (103 weeks): NTP, 1982; reviewed in IARC, 1983. $B6C3F_1$ mice (50/sex/dose) were fed diets containing 0, 50, or 100 mg zearalenone/kg feed for 103 weeks. Among female mice in the high-dose group there was a statistically significant increase in the incidence of hepatocellular adenomas in individual comparisons with the control group (0/50; 2/49; 7/49 for the control, low-, and high-dose groups, respectively; $P < 0.006$). Both male and female mice showed a statistically significant trend in the incidence of pituitary adenomas, and among male and female mice in the high-dose group there was a statistically significant ($P < 0.05$, by Fisher's exact test) increase in the incidence of pituitary adenomas (males: 0/40, 4/45, 6/44; females: 3/46, 2/43, 13/42). NTP concluded that zearalenone should be considered

carcinogenic to B6C3F$_1$ mice of both sexes (pituitary adenomas in males and females; hepatocellular adenomas in females).

2. Rat long-term feeding studies (103 weeks): NTP, 1982; reviewed in IARC, 1983. Fischer 344 rats (50/sex/dose) were fed diets containing 0, 25, or 50 mg zearalenone/kg feed for 103 weeks. No increased incidence of tumors was observed among treated animals. NTP concluded that zearalenone was not carcinogenic to F344/N rats of either sex.

3. Rat long-term feeding studies (up to 104 weeks): Becci *et al.*, 1982. FDRL Wistar rats (50/sex/dose plus 70/sex as controls) were fed zearalenone in diet such that the dose received was 0, 0.1, 1.0, or 3.0 mg/kg-day. After 5 weeks on these diets, animals within each dose group were mated and maintained on zearalenone-containing diet until lactation. F$_1$ generation rats (90/sex/dose plus 140/sex as controls) were maintained on the same levels of zearalenone in feed as the F$_0$ generation. Groups of animals were killed at 13, 26, 64, and 104 weeks and evaluated histopathologically. No increased incidence of tumors was observed among the treatment groups.

Other relevant data

Zearalenone did not produce mutations in five strains of *Salmonella typhimurium*, either with or without metabolic activation, although given the bactericidal activity of the compound the meaning of the findings is questionable (IARC, 1983). In an assay for induction of SCE, chromosomal aberrations, and polyploidy in CHO cells, zearalenone was positive both with and without metabolic activation (Galloway *et al.*, 1987). Zearalenone has been shown to be positive in a *rec* assay in *Bacillus subtilis*, a bacterial DNA repair test (Ueno and Kubota, 1976; Boutibonnes and Loquet, 1979). The *ade2* locus of *Saccharomyces cerevisiae* was unchanged following treatment with two concentrations of zearalenone (Kuczuk *et al.*, 1978). DNA adducts were detected in mouse organs (liver, kidney, ovary), but not rat organs, following oral treatment with zearalenone (Pfohl-Leszkowicz *et al.*, 1995).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenicity concern** over zearalenone because of evidence for the development of hepatocellular adenomas in female mice and pituitary adenomas in both male and female mice. This level of concern regarding the carcinogenicity of zearalenone is supported by the appearance of DNA adducts in experimental animals and evidence for the induction of SCE, chromosomal aberrations, changes in ploidy in cultured animal cells. There is no evidence for tumorigenicity in other experimental animals and data from human exposure are inadequate to draw conclusions. IARC determined that zearalenone is not classifiable as to its carcinogenicity (Group 3; IARC, 1993).

There is a **HIGH** level of **concern over the extent of exposure** to zearalenone. This naturally occurring compound is present in the general food supply to varying degrees, depending on the amount of contamination by fusarium organisms. Still, the data shows that this contamination is far from ubiquitous, and that contaminated foods are much more likely to used as animal feed rather than for human consumption.

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CARCINOGENICITY DATA SUMMARY: 2-BIPHENYLAMINE AND ITS STRONG ACID SALTS

2-Biphenylamine and its strong acid salts, including the hydrochloride (CAS No. 2185-92-4), are chemical intermediates found in the dye, rubber, and pesticide industries. These chemicals have not been evaluated by US EPA or IARC.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to 2-biphenylamine or any of its strong acid salts were identified.

Animal bioassays

1. Mice long-term feed studies: NTP, 1982. Groups of 50 $B6C3F_1$ mice of each sex were administered purified 2-biphenylamine hydrochloride in the diet at two doses (1,000 or 3,000 ppm) for 103 weeks. Hemangiosarcomas were observed in female mice with a statistically significant ($p \leq 0.002$) positive trend (0/49 in the control, 1/50 in low-dose group, and 7/50 in high-dose group). The incidence in the high-dose group was significantly ($p<0.01$) higher than that in controls. The evidence of the increased incidence of hemangiosarcomas in male $B6C3F_1$ mice was less clear. Hemangiosarcoma occurred in male mice with a statistically significant positive trend ($p=0.04$ by a life-table test) with incidences of 0/50 in the control, 2/50 in low-dose group, and 3/50 in high-dose group. None of the pairwise comparisons between treated and control groups was statistically different ($p>0.05$). The development of hemangiosarcomas may have been curtailed in the high-dose group of male mice, since only 21/50 survived until the termination of the study. The hemangiosarcomas found in this experiment are uncommon, incidences in historical controls were 0.7% and 0.9% for female and male mice, respectively. NTP concluded that under the conditions of this bioassay, the chemical was carcinogenic in $B6C3F_1$ female mice and the evidence for carcinogenicity in $B6C3F_1$ male mice was equivocal.

2. Rat long-term feed studies: NTP, 1982. Groups of 49 or 50 Fischer 344/N rats of each sex were administered purified 2-biphenylamine hydrochloride in the diet at two doses (1,000 or 3,000 ppm) for 103 weeks. Inflammatory cells and interstitial fibrosis were found in increased incidence in the kidneys of dosed male rats as compared with controls. In addition, dosed male rats had more focal cellular changes of the liver than did the controls. There were no increased incidences of tumors in rats that can be associated with chemical administration. Under the conditions of this bioassay, NTP concluded that the chemical was not carcinogenic for F344/N rats of either sex. NTP postulated that this may be due to their lack of ability to form the N-hydroxy derivative which is generally viewed as the first step leading to cancer.

Other relevant data

Administration of other nitrogen-containing aromatic compounds such as nitrofen, 4,4'-methylene-bis(2-chloroaniline), and 2-methyl-1-nitroanthraquinone have also been associated with hemangiosarcomas in $B6C3F_1$ mice (NTP, 1982). These three compounds are listed as cancer causing chemicals under Proposition 65. 4-Biphenylamine, a positional isomer of 2-biphenylamine, has been demonstrated to cause cancer in rabbits, dogs, mice, and rats and is designated as a Group 1 (human) carcinogen by IARC (1987). 2-Biphenylamine has been shown to be mutagenic in a number of test systems: several strains of *Salmonella* (with metabolic activation), mouse lymphoma cell forward mutation assay (with metabolic activation), and chromosome aberrations in Chinese hamster ovary cells (without metabolic activation) (Tennant *et al*., 1987). A computerized analysis of structure-activity relationships of the chemical based on a set of rules generated by US EPA experts (Oncologic, version 2.5) indicates that 2-biphenylamine is of low-moderate concern in carcinogenicity.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenicity concern** over 2-biphenylamine and its strong acid salts since 2-biphenylamine hydrochloride has been shown to increase an uncommon cancer, hemangiosarcomas, in female $B6C3F_1$ mice. The concern is reinforced by the fact that 2-biphenylamine hydrochloride is positive in several

genotoxicity tests and is structurally related to a number of nitrogen-containing aromatic compounds that have also been shown to cause cancer in experimental animals.

There is **MEDIUM** level of **concern over the extent of exposure**. 2-Biphenylamine hydrochloride is a chemical intermediate used in the manufacture of C.I. Acid Red 15. It is present as a contaminant in 4-biphenylamine (a rubber anti-oxidant) and in diphenylamine (a dye intermediate, stabilizer for nitrocellulose explosives, and a topical agent for prevention of screwworm infestation in animals.

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CARCINOGENICITY DATA SUMMARY: BROMOETHANE

Bromoethane (ethyl bromide; CAS No. 74-96-4) is primarily used as a chemical intermediate in ethylating reactions in pharmaceutical and chemical industries (ACGIH, 1991). At one time it was also used as a refrigerant, a fruit and grain fumigant, and as an anesthetic, although each of these uses has ceased (NTP, 1989). Bromoethane is currently produced by two companies in the USA. Together these companies manufactured 163.5 million pounds in 1986 (NTP, 1989). No recent import or export information was available in the literature. Furthermore, data on concentrations in the environment were sparse. The limited studies available show bromoethane to be present in very few samples in air, water, and sediments. These data were also not quantified (IARC, 1991). Exposure to bromoethane occurs mostly from breathing contaminated air in the workplace or at waste sites; occupational exposure may also occur via dermal contact (HSDB). It is usually not found in surface water, soil, or food (ATSDR, 1996). Still, this chemical has been found in at least 74 of 1,416 National Priorities List sites identified by the US Environmental Protection Agency (ATSDR, 1996). Bromoethane moves very quickly into the air when released to the environment or when present in soil or water. Once there, it breaks down slowly over several years (ATSDR, 1996).

Bromoethane has been reviewed by IARC (1991) and it was determined there was limited evidence for carcinogenicity in experimental animal and that the compound is not classifiable as to its carcinogenicity to humans (Group 3).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to bromoethane were found in an earlier search by IARC (1991) or more recently by OEHHA.

Animal bioassays

1. Mouse long-term inhalation studies (2 years): NTP, 1989. Female B6C3F$_1$ mice exposed by inhalation to 100, 200, or 400 ppm bromoethane for 6 hr/day, 5 days/wk for two years developed adenomas (1/50; 1/47; 6/48), adenocarcinomas (2/50; 3/47; 19/48), and squamous cell carcinomas (1/50; 1/47; 3/48) of the uterus. No uterine tumors were observed in control animals (0/50). Combined uterine tumors (4/50; 5/47; 27/48) were significantly increased in several dose groups (low-dose, $p = 0.059$; mid-dose, $p = 0.024$; high-dose, $p < 0.001$; Fisher's exact test) and the trend with dose was significant. It was noted that the development of uterine tumors likely decreased the survival of female mice. Male B6C3F$_1$ mice exposed to 0, 100, 200, or 400 ppm bromoethane developed alveolar/bronchiolar tumors (7/50; 6/50; 12/50; 15/50 - adenomas and carcinomas combined). The incidence was significantly elevated in the highest dose group ($p < 0.05$ relative to control, by Fisher's exact test) and the dose trend was significant. The incidence in the high-dose group, however, was within the range of historical controls and there was no evidence of an increased incidence of hyperplasia in support of the finding of neoplasia. NTP concluded there was equivocal evidence for carcinogenic activity for male B6C3F$_1$ mice (lung tumors) and clear evidence of carcinogenic activity for female B6C3F$_1$ mice (uterine tumors).

2. Rat long-term inhalation studies (2 years): NTP, 1989. Pheochromocytomas of the adrenal gland were observed in male F344/N rats exposed for 6 hr/day, 5 days/wk to 0, 100, 200, or 400 ppm bromoethane for two years (8/40; 23/45; 18/46; 21/46). The incidence of this tumor type (combined benign and malignant) was significantly increased in all dose groups ($p < 0.05$, relative to controls by Fisher's exact test). There was no significant increases in the incidences of pheochromocytomas in female rats. A few gliomas were observed in both male and female bromoethane-exposed rats, but not in controls (males: 0/49; 3/50; 0/50; 0/50; females: 0/50, 1/50; 1/48; 3/50). A trend test was significant only in female rats. Granular cell tumors of the brain were observed in male rats only (0/49; 3/50; 1/50; 1/50), but were not found to be significantly increased. Some alveolar/bronchial cell adenomas and carcinomas (combined) were observed in both male and female exposed rats (male: 0/48; 0/49; 4/48; 1/48; female: 0/50; 0/48; 0/47; 3/49). NTP concluded there was some evidence for carcinogenic activity for male F344 rats, and equivocal evidence of carcinogenic activity for female F344 rats.

3. Mouse long-term injection study (8 weeks): Poirier *et al.*, 1975. Groups of male and female A/He mice received 24 intraperitoneal injections (3 times/week) of bromoethane in tricaprylin at 3 dose levels. Surviving animals were killed at 24 weeks and evaluated for lung tumor incidence. The tumor incidence was not significantly elevated over background. The authors stated that metabolic inactivation due to the route of exposure may have been the cause of the negative result.

4. Rat subcutaneous injection study: Dipple *et al.*, 1981; reviewed in IARC, 1991. No significant increase in incidence of tumors was seen among 20 female CB hooded rats after single subcutaneous injection of bromoethane at 1.25, 4.2, or 12.5 mmol/kg body weight. It has been noted that the sample size was small and exposure was limited to a single injection.

Other relevant data

Bromoethane vapor was found to be mutagenic both with and without metabolic activation in a *Salmonella* reverse mutation assay in strains TA100 and TA1535, but not in strain TA98 or in a *Drosophila melanogaster* mutation assay (Barber *et al.*, 1981; Barber *et al.*, 1983; NTP, 1989; IARC, 1991). It was also shown to induce sister chromatid exchange, both with and without activation, but not chromosomal aberrations (Loveday *et al.*, 1989; NTP, 1989). An investigation into an endocrine role in uterine tumor development in mice found no consistent pattern of change in sex hormones as a result of inhalation exposure of mice to bromoethane (Bucher *et al.*, 1995). Bromoethane bears structural resemblance to the known carcinogen chloroethane, which also causes uterine tumors in female mice. A computerized analysis of structure-activity relationships based on a set of rules generated by US EPA experts (Oncologic®, version 2.40) finds that bromoethane is of moderate-to-high concern (*i.e.*, the highest level of concern noted for chemicals which are not included in the database of carcinogenicity bioassay results from which the program rules are derived).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenicity concern** over bromoethane because of clear evidence of carcinogenicity by a likely route of exposure in female mice (uterine tumors), some evidence in male rats (pheochromocytomas) and equivocal evidence in male mice (lung tumors) and female rats (brain and lung tumors). The concern is reinforced by positive findings in short-term mutagenicity and genotoxicity tests and by structural analogy with the known carcinogen chloroethane. IARC has determined that bromoethane is not classifiable as to its carcinogenicity (Group 3; IARC,1991).

There a **MEDIUM** level of **concern over the extent of exposure** to bromoethane. Bromoethane is a moderately high production volume chemical. NIOSH has estimated that approximately 12,000 workers were potentially exposed to bromoethane in the US in 1983 (NIOSH, 1983). The number and volume of uses outside of chemical manufacturing appear to have declined in recent years. Bromoethane does not appear to be present widely in general environmental samples, although it has been detected in some soil, water and air samples from contaminated sites. The compound's volatility and long half-life also support this level of concern over exposure, especially close to waste sites or industrial sources.

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CARCINOGENICITY DATA SUMMARY: β-THIOGUANINE DEOXYRIBOSIDE

β-thioguanine deoxyriboside (9H-purine-6-(1H)-thione; 2-amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)-; β-2'-deoxy-6-thioguanosine; thioguanine deoxyriboside; NCI-C01581; BTGdR; CAS No. 789-61-7), is a derivative of 6-thioguanine and an experimental anticancer drug. The drug was tested in cancer patients who were refractory to other antipurines, but improved responses were not found (Bodey *et al.*, 1976). BTGdR has not been reviewed by IARC. It is not listed in current editions (August 1997) of the Hazardous Substances Data Bank, IRIS, or the Physicians Desk Reference (HSDB, 1997; USEPA, 1997; PDR, 1997).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to βTGdR are available.

Animal bioassays

1. Rat long-term intraperitoneal (ip) injection studies (3 injections/week for 52 weeks + 26 weeks observation): NCI, 1978. Sprague-Dawley rats (35/sex/group) were given ip injections of βTGdR, as the monohydrate, in a buffered saline solution and polysorbate 80 (0.05%) vehicle at 0, 3.5 (LD) or 7.0 (HD mg/kg body weight, three times per week for 52 weeks and then observed for an additional 26 weeks. Toxicity was high and tumor incidences were reported as unadjusted or time-adjusted. Among the female rats, statistically significant increases in malignancies of the ear canal were observed (p=0.008, pooled vehicle control; positive trend test, p=0.004). Among the male rats, statistically significant increases in tumors of the ear canal were also observed (p=0.035, pooled vehicle control) (positive trend test: p=0.014, pooled vehicle control; p=0.046, matched vehicle control). Lymphoma was observed in male rats with positive trend only (p=0.025, pooled vehicle control). NCI concluded that βTGdR in the 0.05% polysorbate vehicle was carcinogenic in female rats and possibly in male rats, based on carcinomas of the ear canal.

2. Mouse long-term ip injection studies (3 injections/week for 52 weeks + 26 weeks observation): NCI, 1978. B6C3F1 mice (35/sex/group) were given ip injections of βTGdR in a buffered saline solution at 0, 2 or 4 mg/kg body weight, three times per week for 52 weeks followed by an additional 27 weeks of observation. No increased tumor incidences were observed among the mice. However, low survival in all mouse groups, perhaps due in part to procedural problems, precluded an unambiguous evaluation of potential treatment related carcinogenicity in this species.

3. Mouse sub-chronic ip injection studies: Stoner *et al.*, 1973 (3 times/week for 8 weeks + 16 weeks observation). A/He (strain A) mice (10/sex/group) were given ip injections of βTGdR, in doses of 0, 0.07, 0.175, and 0.350 g/kg body weight dissolved in 0.1 ml steroid suspending vehicle (SSV, composition not stated) three times per week for a total of 24 doses in 8 weeks. The doses represented 0.2 MTD, 0.5 MTD, and the MTD, where MTD is the maximum tolerated dose. Observation continued up to a total of 24 weeks duration. A direct comparison of lung tumor incidence (mice with tumors/observed mice) is not possible because the incidences for the SSV controls are given for 6, 12, or 24 ip injections, whereas the βTGdR treated mice received 8 injections. According to the authors, the number of lung tumors/mouse among the 0.5 MTD βTGdR group (1.00 ± 0.27) is statistically increased compared to controls (p<0.001).

Other relevant data

BTGdR was selected for testing by NCI to evaluate carcinogenicity of drugs that may be used for humans for prolonged periods. In tests using extracts of human tissues, βTGdR was phosphorylated to the nucleotide triphosphate, which in turn was incorporated into DNA (NCI, 1978). In studies on humans, dogs, monkeys, and mice, toxic responses to βTGdR were observed (Henry *et al.*, 1973; Bodey *et al.*, 1976).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM HIGH** level of **carcinogenicity concern** regarding β-thioguanine deoxyriboside based on the carcinogenic effects observed in male and female Sprague-Dawley rats (NCI, 1978). The levels of concern is reinforced by the observed increases in tumor development during less than lifetime exposure and by the positive

results in male and female A/He (strain A) mice (Stoner *et al*., 1973). Additional support is provided by the observed incorporation of the phosphorylated βTGdR into DNA.

There is **NO IDENTIFIED CONCERN over the extent of exposure** of β-thioguanine deoxyriboside. Clinical studies suggest its use as an antineoplastic agent is limited (Bodey *et al*., 1976). βTGdR is not listed in the current *Physician's Desk Reference* (PDR, 1997). A recent OEHHA search found no indication that the drug is used or produced in the USA at the present time.

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CARCINOGENICITY DATA SUMMARY: *BIS*(TRI-*N*-BUTYLTIN)OXIDE

Bis(tri-*n*-butyltin)oxide (tri-*n*-butyltin oxide; CAS No. 56-35-9) is primarily used as an antifoulant paint additive on ship and boat hulls to discourage the growth of marine organisms such as barnacles, mussels and algae. *Bis*(tri-*n*-butyltin)oxide and other tributyltin compounds are also used as wood preservatives, disinfectants and biocides for use in paper and pulp mills, cooling towers, breweries, textile mills, and leather-processing facilities. US EPA (1997) assigned tributyltin oxide to category D or to the "cannot be determined" category for carcinogenicity.

Carcinogenicity Data available:

Epidemiological studies

An epidemiological study of workers occupationally exposed to wood treating chemicals including chromated copper arsenate, tributyltin oxide and pentachlorophenol did not indicate adverse health effects or increased incidence of mortality and cancer rates (Gilbert *et al*., 1990).

Animal bioassays

1. Rat long-term feed studies: Wester *et al*., 1990. Groups of 50 male and female Wistar rats were fed 0, 0.5, 5 or 50 mg *bis*(tri-*n*-butyltin)oxide/kg diet for 2 years. The incidence of benign pituitary tumors was increased in high-dose females (controls, 22/50; low-dose, 32/50; medium-dose, 22/50; high-dose, 35/50; $p<0.01$ versus controls) and in high-dose males (controls, 34/50; low-dose, 39/50; medium-dose, 29/50; high-dose, 43/50; $p<0.01$ versus controls). Some of the pituitary tumors were considered to be fatal when rats showed clinical signs of central nervous dysfunction and showed at autopsy large pituitary tumors. Analysis of these fatal tumors showed a statistically significant increase in the males and females fed 50 mg bis(tri-*n*-butyltin)oxide/kg diet and in males fed 0.5 mg/kg. There was also a statistically significant increase ($p<0.001$) of pheochromocytomas of the adrenal medulla in both sexes fed 50 mg/kg (males: 33/50 vs. 16/50 in controls; females: 34/50 vs. 3/50 in controls). There was no evidence of increased progression: minimal signs of malignancy were seen in less than 20% of these tumors, and metastases were not seen. A highly malignant pancreatic tumor occurred in two females from the 50 mg/kg group and one from the 0.5 mg/kg group. They were diagnosed as anaplastic carcinomas and are uncommon in rats (incidence varies between 0 and 1.1% in strains from various laboratories). There is also evidence indicating that in the 50 mg/kg group, the chemical produced parathyroid adenomas in males.

2. Mice long-term feed studies: summarized and reviewed by CDPR, 1995. Groups of 50 male and female CD-1 mice were fed with 0, 5, 25 or 50 ppm *bis*(tri-*n*-butyltin)oxide in the diet for 18 months. No significant increases in tumor incidence were observed in the study.

Other relevant data

Bis(tri-*n*-butyltin)oxide gave mostly negative results in short-term genotoxicity tests (Davis et al., 1987). However it was positive in the chromosomal aberration test using Chinese hamster ovary cells, the mouse micronucleus test, and the *S. typhimurium* strain TA100 test, with metabolic activation (Davis et al., 1987). It was also shown to cause DNA damage in the rec-assay (Hamasaki et al., 1992). Degradation products of bis(tri-*n*-butyltin)oxide such as di-*n*-butyltin and mono-*n*-butyltin were shown to be genotoxic in the rec-assay and the SOS chromotest (Hamasaki et al., 1992). In addition, there is evidence indicating that tri-*n*-butyltin enhanced the induction of breakage-type chromatid aberrations by clastogens (Sasaki *et al*., 1993).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM** level of **carcinogenicity concern** for bis(tri-*n*-butyltin)oxide, since tumors of the pituitary and adrenal glands were observed in both male and female rats. In the high-dose groups of both sexes, about a third of the pituitary tumors caused early mortality and about 15% of the adrenal gland tumors were found to be malignant. The concern is reinforced by the observations of genotoxicity of bis(tri-*n*-butyltin)oxide and its degradation products in short-term tests. US EPA (1997) assigned tributyltin oxide to category D or to the "cannot be determined" category.

There is **HIGH** level of **concern over the extent of exposure**. *Bis*(tri-*n*-butyltin)oxide is the major tributyltin-containing pesticide, and is widely used as an antifoulant, wood preservative, disinfectant and biocide. US EPA estimated that about 600,000 gallons of organotin-containing anti-foulant paint are sold annually (Pesticide and Toxic Chemical News, 1987). Bis(tri-*n*-butyltin)oxide's pesticidal action is dependent upon its degradation and release of tri-*n*-butyltin. Tri-*n*-butyltin has been detected at ppb levels in San Diego Bay, San Francisco Bay, Los Angeles/Long Beach Harbor, and the Great Lakes (Pesticide and Toxic Chemical News, 1987). The potential for humans to be exposed to this chemical is high as it is accumulated in oysters, mussels, crustaceans, mollusks, and fish. The chemical is extremely toxic to crustaceans and may affect the growth and development of aquatic organisms.

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CARCINOGENICITY DATA SUMMARY: DICOFOL

Dicofol (CAS No. 115-32-2, also known as 2,2,2-trichloro-1,1-bis(4-chlorphenyl)-ethanol, and Kelthane®) is a chlorine-containing pesticide, and is used as an acaricide on a wide variety of fruit, vegetable, ornamental, and field crops. It is manufactured from DDT. In 1986, use of Dicofol was temporarily canceled by the US EPA because of concerns raised by high levels of DDT contamination (Extension Toxicology Network, 1993). However, modern manufacturing processes can produce technical grade Dicofol which contains less than 0.1 % DDT, so use and production was allowed to continue.

Dicofol is practically insoluble in water and adsorbs very strongly to soil particles. It is therefore nearly immobile in soils and unlikely to infiltrate groundwater. Even in sandy soil, Dicofol was not detected below the top 3 inches in standard soil column tests (Extension Toxicology Network, 1993). Still, it is possible for Dicofol to enter surface waters when soil erosion occurs, and it has been detected in groundwater (Extension Toxicology Network, 1993; Howard, 1991). After entry into water, Dicofol is degraded by hydrolysis to dichlorobenzophenone (Extension Toxicology Network, 1993). In addition, although residues in soil decrease rapidly, traces may remain for more than a year (Worthing, 1987). Moreover, a number of studies have shown that Dicofol residues on treated plant tissues can remain unchanged for up to 2 years (Extension Toxicology Network, 1993). An estimated 2 to 2.5 million pounds of Dicofol is applied annually in the United States, primarily for mite control by the cotton and citrus industries. In California, almost 590 thousand pounds were applied in 1993. Daily intake values based on various food surveys ranged from 0 to 4.8 mg/kg, with the majority of the samples indicating intake values below 1 mg/kg (Howard, 1991). Dicofol has also been detected in the well water of some California communities (Howard, 1991).

Dicofol was evaluated by IARC (1983, 1987), which found limited evidence of carcinogenicity in animals (IARC group 3). US EPA had earlier listed Dicofol as a group B2 carcinogen, but now prefers to classify it as group C (Larkin, 1989): the IRIS entry has been withdrawn pending further evaluation (IRIS, 1997) .

Carcinogenicity data available:

Epidemiological studies

No reports of studies of the effects of long-term human exposure to Dicofol were identified by IARC (1983), or in a recent search of the scientific literature by OEHHA.

Animal bioassays

Bioassays of Dicofol in rats and mice were undertaken by NCI (1978), and by Rohm and Hass Co. Toxicology Department. The latter study has not been published but was reviewed by CDPR (1992). CDPR also describes some other chronic toxicity studies in rats, mice and dogs; however these were not considered suitable in design or reporting detail for assessment of possible carcinogenicity.

1. Mouse feeding studies (78 weeks + 14-15 weeks observation): NCI, 1978. $B6C3F_1$ male and female mice (50/sex/dosed group, 20/sex controls) were given dicofol in feed. Diet containing test material was fed for 78 weeks at concentrations which were increased during the course of the study because of the apparent development of increased tolerance. Time-weighted average concentrations in the diet were 0, 264, 528 ppm for males and 0, 122, 243 ppm for females. At the end of the dosing period the animals were observed for a further 14 (males) or 15 (females) weeks while fed plain diet. Hepatocellular tumors occurred in male mice in 3/18 controls, 22/50 low-dose ($p=0.035$), and 35/47 high-dose animals ($p <0.001$). The NCI report identifies these tumors as carcinomas, although some commentators have argued that they were adenomas (CDPR, 1992). In exposed female mice, no hepatocellular tumors were observed, and there were no statistically significant increases in tumors at other sites. IARC (1983) noted that the sizes of the control groups were inadequate according to current recommendations for study design.

2. Rat feeding studies (78 weeks + 34 weeks observation): NCI, 1978. Osborne-Mendel rats (50/sex/dosed group, 20/sex controls) were given dicofol in feed for 78 weeks. The dietary concentration was increased during the treatment period for males, resulting in time-weighted averages of concentrations of 0, 471 and 942 ppm. The females were given diet containing 0, 380 or 760 ppm Dicofol throughout the dosing period. At the end of the

dosing animals were observed for a further period of 34 weeks on plain diet. No statistically significant development of tumors was noted in rats. IARC noted the short treatment period for male and female rats and inadequate sizes of the control groups according to current recommendations for study design (IARC, 1983).

3. Rat feeding studies (24 months): Rohm and Haas Co. Toxicology Department, evaluated by CDPR (1992). Male and female Crl-CD rats (100/sex/group) were fed 0, 4.52, 45.32 and 238.7 ppm Dicofol for 24 months: interim sacrifices of 10 animals/group were made at 3, 12, and 18 months. Liver hypertrophy, hepatocellular necrosis and vacuolation of adrenal cortical cells were observed in the two higher dose groups, but no increases in tumor incidence were reported.

Other relevant data

Dicofol has been reported not to be mutagenic towards *Bacillus subtilis*, *Escherischia coli*, and *Salmonella typhimurium*, with or without metabolic activation. Dicofol has been reported to be negative in the Chinese hamster lung chromosomal aberration test and positive in the rat bone-marrow micronucleus test (IARC, 1983). CDPR (1992) reviewed several other genotoxicity studies; Dicofol was negative in tests for gene mutation in *Salmonella* and Chinese hamster ovary (CHO) cells and for unscheduled DNA synthesis in CHO cells. Results of a cytogenetic study *in vitro* in CHO cells were equivocal; a cytogenetic study *in vitro* in rats was negative.

Dicofol is a structural analog of DDT, a known carcinogen, and has many similar chemical and toxicological properties. Dicofol may be metabolized into DDE (NCI, 1978), but this is not proven; the DDE residues observed may have been derived from impurities in the dosed material (Hayes and Laws, 1991). Dicofol induces various cytochrome P-450 enzymes in rats and mice given dicofol by the oral or intraperitoneal routes (Narloch *et al.*, 1987; CDPR, 1992). In a thirteen week feeding study (Flodstrom *et al.*, 1990) in partially hepatectomized male Sprague-Dawley rats initiated with N-nitrosodiethylamine, statistically significant increases in GGT-positive hepatic foci ($p<0.05$) were observed in the 1000 ppm dose group. Inhibition of gap junctional communication by Dicofol was also studied by Flodstrom *et al.* (1990) in V79 Chinese hamster cells and WB-F344 rat liver epithelial cells. Dicofol may be a tumor promoter; the mechanism is unknown but it was suggested by the authors that this results from its effects on gap junctional communication.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM** level of **carcinogenicity concern** over Dicofol since liver tumors have been observed in male mice and it has close structural and toxicological similarity to DDT, a known carcinogen with a similar pattern of tumor induction. The fact that carcinogenicity of Dicofol was not observed in female mice or in rats, and the consistently negative genotoxicity data may indicate that a non-standard mechanism is responsible for the effect in male mice.

There a **HIGH** level of **concern over the extent of exposure** to Dicofol. As a result of its use as a pesticide, dicofol has been shown to occur widely in food, soil, and water. While studies have demonstrated relatively small exposures to the general population, the number of people exposed is high. In addition to the general population exposure, occupational exposure can occur in the manufacturing of the chemical or during its application to crops. Extensive use of, and thus exposure to, Dicofol appears to occur in California.

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CARCINOGENICITY DATA SUMMARY: DI(2-ETHYLHEXYL)ADIPATE

Di(2-ethylhexyl)adipate (DEHA; dioctyl adipate; DOA; CAS No. 103-23-1) is a plasticizer which is added to vinyl plastics to impart low-temperature flexibility. It has also been used as a lubricant, functional (hydraulic) fluid, and a solvent for consumer products such as cosmetics. The most important use of DEHA is in the polyvinyl chloride plastic wraps used for meats, where DEHA may comprise up to 40% of the product (IARC, 1982). Leaching to foods, particularly those high in fat or alcohol, has been demonstrated. Indoor air sampling in supermarkets where DEHA-containing food wrap is cut by hot-wire has shown levels as high as 0.14 mg/m^3 in the immediate vicinity of the cutting (NTP, 1981; citing Vandervort and Brooks, 1977). DEHA has also been shown to leach from polyvinyl chloride tubing into human plasma during hemodialysis (NTP, 1981). Estimates from the California TRI of releases of DEHA totaled approximately 44,000 lb in 1994. US production of DEHA has been estimated at 1.25×10^7 kg for 1984, with export estimates of 7.54×10^5 kg for the same year (HSDB, 1994).

US EPA (1993) has classified DEHA as a possible human carcinogen (group C). IARC (1982, 1987) has determined that limited evidence of carcinogenicity in animals leaves this compound unclassifiable as to its carcinogenicity (Group 3; IARC, 1982; IARC, 1987).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to DEHA were found in an earlier search by IARC (1982) or in a recent literature search by OEHHA.

Animal bioassays

1. Mouse long-term diet studies (103 weeks): NTP, 1981. Hepatocellular adenomas and carcinomas were observed in $B6C3F_1$ mice treated with two levels of DEHA (12,000 or 25,000 ppm) in their diet for 103 weeks. Among male mice, the incidence of combined liver adenoma or carcinomas was increased significantly in the high-dose group (13/50 - control, 20/49 - low-dose, 27/49 - high-dose; P = 0.03, by Fisher's exact test). A trend test for these tumors was also statistically significant, however, it was noted that the high-dose tumor incidence did not differ significantly from the incidence among historical controls in the laboratory and there was no difference in time-to-tumor among the animals in this experiment (IRIS, 1993). The incidences of hepatocellular carcinomas among males were 7/50, 12/49, and 12/49 for the control, low-, and high-dose groups, respectively. Among female mice, the incidence of hepatocellular carcinoma was significantly increased among mice in both the low- and high-dose groups (1/50, 14/50, 12/49; P < 0.001, by Fisher's exact test). For these tumors, there was also a statistically significant trend test, and time-to-tumor was significantly decreased in treated animals. It was noted that the two batches of the compound used in the investigation contained at least five or seven unidentified impurities which were found by gas-liquid chromatography. NTP concluded that DEHA was carcinogenic to female $B6C3F_1$ mice (hepatocellular carcinoma), and probably carcinogenic to male $B6C3F_1$ mice (combined hepatocellular adenoma and carcinoma).

2. Rat long-term diet studies (103 weeks): NTP, 1981. F344 rats exposed to two concentrations of DEHA in feed (12,000 or 25,000 ppm) for 103 weeks did not develop increased incidences of any tumors over control animals. As noted above, two batches of compound in the study contained unidentified impurities. NTP concluded that DEHA was not carcinogenic for F344 rats.

3. Mouse injection and skin-painting studies: Hodge *et al.*, 1966; as described in IRIS, 1993. No tumors (with the exception of one fibromyxoma in a male mouse) were observed in C3H/Anf mice (50/sex/group) injected once subcutaneously with 0.1 mg DEHA in trioctanoin. Skin-painting of mice (50/sex/group) once weekly with 0.1 mg DEHA in 20 mL acetone or 10 mg DEHA in 50 mL acetone produced no evidence of application site tumors in mice. The maximum total dose was reported to be 8.8 and 920 mg DEHA for males and 9.8 and 1010 mg DEHA for females, suggesting that application continued for approximately 2 years.

4. Rat and dog long-term diet studies (1 or 2 years): unpublished studies reported by Hodge *et al.*, 1966; as described in IRIS, 1993. Rats (numbers not presented) were fed DEHA in their diet (0, 0.1, 0.5, or 2.5%) for 2

years and dogs (2-4/group) were fed 0, 0.07, 0.15 or 0.2% DEHA for 1 year. No evidence of dose related tumor induction was described by the authors. The size and nature of the studies and the level of detail in the study descriptions were insufficient to permit any conclusions to be drawn from this report.

Other relevant data

DEHA was not found to be mutagenic either with or without metabolic activation in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, or TA100 (Barber *et al.*, 1985; Simmon *et al.*, 1977; Zeiger *et al.*, 1982). DEHA was not genotoxic in a mouse lymphoma L5178Y assay, unscheduled DNA synthesis assay in rat liver cells, and in a mouse micronucleus assay (Barber *et al.*, 1985). Intraperitoneal injection of DEHA in mice produced dominant lethal mutations during spermatogenesis after a single dosing (Singh *et al.*, 1985). DEHA belongs to a class of plasticizers and other compounds which induce peroxisome proliferation in the liver. Peroxisome proliferation inducers, including the DEHA analogues di(2-ethylhexyl)phthalate, tris(2-ethylhexyl)phosphate, and 2-ethylhexyl sulphate, are associated with a number of toxic effects, including oxidative damage to DNA and other cell components, activation of cell proliferation which may occur via a specific receptor, and liver carcinogenesis in rats or mice (Kluwe *et al.*, 1985; Kluwe, 1986, IARC, 1995). DEHA was found to be the least potent among a number of plasticizers tested for peroxisome proliferation activity (US EPA, 1986; US EPA, 1987; Lin, 1987).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM** level of **carcinogenicity concern** over di(2-ethylhexyl)adipate because of evidence of dose-related liver tumor development in both sexes of one species of animal (mouse). There was no evidence of tumor development in well-conducted studies of male and female rats. This level of concern is supported by evidence that DEHA produces dominant lethal mutations during spermatogenesis after a single injected dose in mice. DEHA bears a structural resemblance to other compounds associated with liver carcinogenesis in rats and mice. IARC has determined that DEHA is not classifiable as to its carcinogenicity (Group 3; IARC, 1982; IARC, 1987).

There is a **HIGH** level of **concern over the extent of exposure** to di(2-ethylhexyl)adipate. There is a great potential for contamination of food because DEHA is not chemically bound in products such as plastic food wrap which results in leaching of the compound and potential oral exposures. The presence of DEHA in indoor air environments where DEHA-containing plastics are used has also been demonstrated, resulting in potential inhalation exposures. NIOSH has estimated that approximately 8000 workers may be occupationally exposed to DEHA (HSDB, 1994).

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CARCINOGENICTY DATA SUMMARY: PATULIN

Patulin (4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one; CAS No. 149-29-1) is a mycotoxin produced by a number of fungi (e.g., several *Penicillium* species) and is commonly found in contaminated fruits. This unsaturated heterocyclic lactone has been identified in rotten apples contaminated by fungi and in commercial sweet apple cider at levels up to 45 mg/L (IARC, 1976). It has also been identified in numerous other moldy fruits, vegetables, cereals, and animal feeds; it is stable in apple and grape juices and in dry corn (Smith *et al*., 1993). In almost all cases, however, patulin contamination has been limited to rotting or moldy foods (IARC, 1986). It has both bacteriostatic and bactericidal effects and is effective against various gram-negative and gram-positive bacteria. Patulin is not produced commercially, and is not authorized for use as a drug by the US Food and Drug Administration (IARC, 1976). It is available from one company in the US for experimental purposes only.

Patulin was reviewed by IARC in 1976, 1985, and 1987. IARC (1987) classified patulin as a Group 3 substance, concluding that there are no data available on the carcinogenicity in humans and that there is inadequate evidence of carcinogenicity in experimental animals. The studies reviewed by IARC, as well as several studies published since IARC's review are briefly described below.

Carcinogenicity Data available:

Epidemiological studies

No studies of the long-term health effects of human exposure to patulin were identified in the published literature by IARC (1987) or in recent literature searches performed by OEHHA.

Animal bioassays

1. Rat subcutaneous (s.c.) injection study (2X/wk for 61 or 64 weeks): Dickens and Jones, 1961, as reviewed by IARC, 1976. Two groups of 5 male Wistar rats were given s.c. injections of 0.2 mg patulin in arachis oil/rat, 2X/wk for 61 or 64 weeks. Another group of 5 males received 2.0 mg patulin/rat at the same dosing schedule, but all animals in this high-dose group died and could not be evaluated. Four out of four rats in the first group and 2/4 rats in the second group developed local sarcomas. No injection-site tumors were seen in 16 vehicle controls observed for 54-107 weeks. IARC (1985) noted the small number of animals used.

2. Mouse *in utero* gavage study (2X/day on gestation days 14-20): Osswald *et al*., 1978, as reviewed by IARC, 1985. Two groups of 12 female pregnant Swiss mice were given by gavage 0 or 2 mg patulin/kg body weight 2X/day on gestation days 14-20. The dams and offspring (controls: 40 males, 54 females; treated: 35 males, 41 females) were observed for life. No increased incidences of tumors were observed in either the treated dams or their offspring. IARC (1985) noted the small number of animals used.

3. Rat gavage study (2X/wk for 64 weeks, observed up to 110 weeks): Osswald *et al*., 1978, as reviewed by IARC, 1985. Two groups of 50 female Sprague-Dawley rats were given by gavage 0 or 1 mg patulin/kg body weight 2X/week for 4 weeks, for the next 60 weeks the dose in the treated group was increased to 2.5 mg patulin/kg body weight. Animals were observed for 110 weeks. No treatment-related increased incidence of tumors was observed. IARC (1985) noted the use of only one dose group.

4. Rat *in utero*/long-term gavage studies (gavage to F_0 generation from 4 weeks prior to mating through lactation, and gavage of F_1 generation 3X/wk for 6, 12, 18 or 24 months): Becci *et al*., 1981, as reviewed by IARC, 1985. Groups of 70 male and female Wistar rats which had been exposed *in utero* to patulin (administered to the F_0 generation by gavage at 0.1, 0.5 or 1.5 mg/kg body weight 4 weeks prior to mating, continuing through gestation and lactation) received either 0.1, 0.5, or 1.5 mg patulin/kg body weight by gavage 3X/week for 6, 12, 18, or 24 months. Two groups of 110 male and female offspring served as controls. No treatment-related increased incidence of tumors was observed. IARC (1985) noted the incomplete reporting of tumor incidences in the study.

Other relevant data

Patulin induced DNA damage in *Bacillus subtilis* (IARC, 1985), rat fibroblasts, and hamster ovary cells (Stetina and Votava, 1986), but not in *Escherichia coli* (IARC, 1985). It was not mutagenic to *Salmonella typhimurium* (IARC, 1985; Wurgler *et al*., 1991; Lindroth and von Wright, 1990), nor was it genotoxic in the SOS microplate assay (Sakai *et al*., 1992). It induced mutations (Sumbu *et al*., 1983; IARC, 1985) but not mitotic recombination in *Saccharomyces cerevisiae* (IARC, 1985). Patulin also caused mutations in *Drosophila melanogaster* (Belitsky *et al*., 1985).

It induced DNA strand breaks in HeLa cells (IARC, 1976) and *Escherichia coli* (Lee and Roschenthaler, 1986), but not unscheduled DNA synthesis in cultured mammalian cells. It induced sister chromatid exchanges (SCEs) in cultured mammalian cells (IARC, 1985). It induced chromosomal aberrations (Roll *et al*., 1990) but not SCEs in bone marrow cells of Chinese hamsters treated *in vivo* (IARC, 1985). It induced chromosome aberrations in cultured human peripheral leucocytes (IARC, 1976). It did not induce dominant lethal mutations in rats or mice (IARC, 1985).

It also caused DNA synthesis inhibition in rat fibroblasts, hamster ovary cells (Stetina and Votava, 1986), and human HeLa cells (Kawasaki *et al*., 1972). Patulin inhibited protein and RNA synthesis in rat alveolar macrophages *in vitro* (Sorenson *et al*., 1985).

Patulin significantly increased the number of hyperplastic liver nodules when administered in the diet at 4 mg/kg for six weeks to partially hepatectomized male Fischer 344 rats pretreated with carbon tetrachloride and 2-acetylaminofluorene (Imaida *et al*., 1982).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM** level of **carcinogenicity concern** for patulin, based on positive findings in several genotoxicity assays. Consistent with this level of concern, local tumors were observed in a single rat injection study that was limited by extremely small sample size (n=5/group) and only one evaluable dose level. Other studies in rats and mice by relevant routes were nonpositive, however, all studies in animals were non-standard in design and all but one administered the test compound for less than lifetime. IARC (1985) concluded that there was inadequate evidence for the carcinogenicity of patulin in experimental animals.

There is a **HIGH** level of **concern over the extent of exposure** to patulin since consumption of contaminated foods by the general population may occur. No data were identified on historical or current levels in the US food supply; it is unclear how frequent such contamination is. Patulin is produced commercially in small quantities for experimental research purposes.

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CARCINOGENICITY DATA SUMMARY: PENTACHLORONITROBENZENE

Pentachloronitrobenzene (PCNB; quintozene; CAS No. 82-68-8) is a halogenated benzene derivative and agricultural pesticide. It is used as a chemical intermediate, herbicide, fungicide for seed treatment and soil fumigation, and as a slime inhibitor in industrial waters (Howard, 1991). As a fungicide, it is used for crops such as cotton, peanuts, barley, corn, oats, peas, wheat, and rice. It is also used for vegetables such as beans, broccoli, lettuce, and potatoes; approximately 2 million pounds are used annually in the United States for agricultural purposes (NTP, 1987). In 1995, 109,920 pounds were used in California alone (CDPR, 1996). Through its use as a fungicide, pentachloronitrobenzene has the potential to contaminate the food, soil, and water of the general population. In addition, occupational exposure can occur during manufacture and application to soils and crops. It has been detected in drinking water, in California well water, and in soil (Howard, 1991). In one 1972 study, US cropland soil levels ranged from 0.98 to 2.61 ppm (Howard, 1991). In a 1977 analysis of adult total diet samples from 20 US cities, residues were detected in 15 out of 20 oil, fats and shortening composite samples (Howard, 1991). It has been detected in potatoes at 0.1 ppm; in endive leaves and roots at 0.06-83 ppm; and in cows' milk at 0.001-0.01 ppm (IARC, 1974). It also been detected in cheese, fruits, vegetables, nuts, and oilseed by-products (Howard, 1991).

The US EPA has classified pentachloronitrobenzene as a group C carcinogen, based on limited evidence in animals and no data in humans (US EPA, 1995). IARC initially reviewed this chemical in 1974; in 1987, IARC classified pentachloronitrobenzene as a group 3 carcinogen, concluding that there was no evidence of carcinogenicity in humans and limited evidence of carcinogenicity in animals (IARC, 1987). The IARC (1987) review did not include the several animal bioassays available since 1974.

Carcinogenicity Data available:

Epidemiological studies

No studies of the long-term effects of human exposure to pentachloronitrobenzene have been reported.

Animal bioassays

1. Rat long-term feeding studies (78 weeks + 33-35 weeks observation): NCI, 1978. Groups of 50 male and 50 female Osborne-Mendel rats were given pentachloronitrobenzene (contaminated with 1% hexachlorobenzene) in feed for 78 weeks. Time-weighted concentrations were 5,417 ppm and 10,064 ppm for low- and high-dose males and 7,875 ppm and 14,635 ppm for low- and high-dose females. The NCI concluded that under the conditions of this bioassay, pentachloronitrobenzene was not carcinogenic in male or female Osborne-Mendel rats.

2. Rat long-term feeding studies (104 weeks): unpublished, as reviewed by Santodonato *et al*., 1985, and International Programme on Chemical Safety, 1984. Groups of 50 male and 50 female Wistar rats were fed 0, 100, 400, or 1200 ppm pentachloronitrobenzene for 104 weeks. No treatment-related tumors were observed in males or females. The pentachloronitrobenzene used in these studies was contaminated with 2.7% hexachlorobenzene, a known carcinogen.

3. Rat long-term feeding studies (2 years): unpublished, as reviewed by CDPR, 1993; US EPA, 1995. Pentachloronitrobenzene (99.4%) was given at concentrations of 0, 20, 2000, or 6000 ppm to 60 Charles River CD rats/sex/group for 2 years. Thyroid tumors (follicular adenoma and carcinoma) were observed in both sexes. A statistically significant increase in thyroid follicular cell adenomas was observed by both pair-wise and trend analyses in males; and a positive trend was observed in females.

4. Mouse long-term feeding studies (78 weeks + 14-15 weeks observation): NCI, 1978. Groups of 50 male and 50 female $B6C3F_1$ mice were given pentachloronitrobenzene (contaminated with 1% hexachlorobenzene) in feed for 78 weeks. Time-weighted concentrations were 2,606 ppm and 5,213 ppm for low- and high-dose males and 4, 093 ppm and 8,187 ppm for low- and high-dose females. The NCI concluded that pentachloronitrobenzene was not carcinogenic in male or female $B6C3F_1$ mice. Limitations of this study included poor survival in male mice and incomplete examination of livers from dosed female mice.

5. Mouse long-term feeding studies (103 weeks): NTP, 1987. Groups of 50 male and 50 female $B6C3F_1$ mice were administered diets containing 0, 2,500, or 5,000 ppm pentachloronitrobenzene for 103 weeks. No compound-related neoplastic lesions were observed in either sex. The NTP concluded that there was no evidence of carcinogenicity of pentachloronitrobenzene in male or female $B6C3F_1$ mice.

6. Mouse long-term feeding studies (104 weeks): unpublished, as reviewed by Santodonato *et al.*, 1985, and International Programme on Chemical Safety, 1984. Groups of 100 male and 100 female Swiss SPF mice were fed 0, 100, 400, 1200 ppm pentachloronitrobenzene for 104 weeks. An increase in subcutaneous fibrosarcomas was observed in females. The incidence was 0/90 in controls and 12/91 in animals fed 1200 ppm pentachloronitrobenzene ($p < 0.0001$ by Fisher's Exact Test). No treatment-related tumors were observed in males. The pentachloronitrobenzene used in these studies was contaminated with 2.7% hexachlorobenzene, a known carcinogen. However, hexachlorobenzene has not been reported to induce subcutaneous fibrosarcomas by the oral route in female Swiss mice (US EPA, 1997).

7. Mouse long-term gavage/feeding studies (78 weeks): Innes *et al*., 1969. Pentachloronitrobenzene (technical grade, containing 11% hexachlorobenzene) was given by gavage on days 7-28 at 464 mg/kg to groups of 18 $B6AKF_1$ mice/sex and groups of 18 $B6C3F_1$ mice/sex, and thereafter in the diet at 1,206 ppm until age 78 weeks. Treatment-related increases in liver tumors were observed in male $B6AKF_1$ mice (10/17 vs. 5/90 controls, $p < 0.001$) and female $B6C3F_1$ mice (4/18 vs. 0/87 controls, $p < 0.001$). The small numbers of animals used and the presence of 11% hexachlorobenzene, a known rodent liver carcinogen, in the test substance limit the usefulness of this study.

Additional studies not reviewed here include a skin painting study in mice, early feeding studies in rats and mice where either the study design or the presence of high levels of hexachlorobenzene as an impurity in the test chemical severely limit the interpretation of the findings, and multiple non-lifetime (i.e., one- or two-year) feeding studies in dogs.

Other relevant data

Pentachloronitrobenzene was mutagenic in *E. coli* in one study, but tested negative in two other studies (NCI, 1978). Pentachloronitrobenzene induced chromosomal aberrations, but not sister-chromatid exchanges in CHO cells (NTP, 1987). Pentachloronitrobenzene induced chromosomal nondisjunction in *Aspergillus nidulans* (Morpurgo *et al*., 1979) and unrepairable damage to the DNA of *Salmonella typhimurium* (Rashid and Mumma, 1986). Pentachloronitrobenzene was not mutagenic in *Drosophila melanogaster* (NCI, 1978), *Salmonella typhimurium*, *Bacillus subtilis*, *S. cerevisiae*, or the L5178 tk+/- mouse lymphoma assay, and tested negative in a dominant lethal test with mice and in a UDS assay in human fibroblast WI-33 cells (NTP, 1987).

A computerized analysis of structure-activity relationships based on a set of rules generated by US EPA experts (Oncologic®, version 2.40) finds that pentachloronitrobenzene is of low-to-moderate concern.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM** level of **carcinogenicity concern** over pentachloronitrobenzene since thyroid follicular cell adenomas and carcinomas were observed in both sexes of one strain of rats, however, this was not observed in two other rat strains. Treatment-related increased tumor incidences were reported in mice (liver tumors in both sexes in one study and subcutaneous fibrosarcomas in females in another), however, the test chemical administered in these studies contained >1% hexachlorobenzene, a known rodent liver carcinogen, as an impurity. The mixed results from numerous short-term tests for genetic toxicity neither raises nor lowers the level of concern. A computerized analysis of structure-activity relationships based on a set of rules generated by US EPA experts (Oncologic®, version 2.40), which predicts that pentachloronitrobenzene is of low-to-moderate concern, is consistent with the level of concern. The US EPA (1995) and IARC (1987) classified this chemical as group C and group 3, respectively.

There a **HIGH** level of **concern over the extent of exposure** to pentachloronitrobenzene since large amounts are used in California agriculture. The general population is likely to be exposed through ingestion of residues in food

crops, milk from cows fed contaminated feed, and water. Occupational exposure may occur during manufacture, formulation, and application of this fungicide.

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CARCINOGENICITY DATA SUMMARY: PIPERONYL SULFOXIDE

Piperonyl sulfoxide (CAS No. 120-62-7) is used to enhance the insecticidal properties of the pyrethrins by inhibiting pyrethrin detoxification enzymes, probably microsomal oxidases, in the insect. The chemical is no longer used in pesticide products registered in California.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Mice long-term feed studies: NCI, 1979. Groups of 50 $B6C3F_1$ mice of each sex were administered technical-grade piperonyl sulfoxide in the diet at two doses for 104 or 105 weeks. The doses for the males were 350 and 700 ppm. Due to excessive weight depression in the dosed female mice, the doses for this sex were reduced after week 20. The time-weighted average doses for the females were 295 and 754 ppm. There was a statistically significant dose-response relationship (p less than 0.001) between the administered dose and hepatocellular carcinomas in males (control 6/18, low-dose 31/50, high-dose 46/50). Although hepatocellular carcinomas were also found in 1/19 control females, 3/50 low-dose females, and 6/50 high-dose females, the pairwise comparisons between treated and control animals and trend tests were not statistically significant. NCI concluded that under the conditions of this bioassay, piperonyl sulfoxide was not carcinogenic in female $B6C3F_1$ mice but was carcinogenic in male $B6C3F_1$ mice, increasing the incidence of hepatocellular carcinoma.

2. Rat long-term feed studies: NCI, 1979. Groups of 50 Fischer 344 rats of each sex were administered technical-grade piperonyl sulfoxide in the diet at one of several doses, either 1,500 or 3,000 ppm for the males and either 3,000 or 6,000 ppm for the females, for 105 weeks. There was an increased incidence of malignant lymphoma in the dosed rats when compared with controls. However, this is a frequently occurring neoplasm in aged Fischer 344 rats, and the incidence observed in this study does not exceed that of historical controls. Further, the average age at which the neoplasm was observed is not different in dosed and control rats in this study. NCI concluded that tumor incidences observed among the dosed groups were not significantly higher than those of the control groups, and piperonyl sulfoxide was not carcinogenic under the test system.

Other relevant data

Certain structural congeners of piperonyl sulfoxide, such as safrole, isosafrole, and dihydrosafrole, have been reported to be carcinogenic in rats and mice, inducing tumors of the liver, esophagus, or lung, depending on the species and sex (NCI, 1979). Piperonyl sulfoxide has been shown to be mutagenic in mouse lymphoma assay, with metabolic activation. It was not genotoxic in the *Salmonella* reversion assay, the chromosomal aberration assay using Chinese hamster ovary cells, and sister-chromatid exchange test using Chinese hamster ovary cells, with or without enzymatic activation (Caspary *et al*., 1988).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **MEDIUM** level of **carcinogenicity concern** over piperonyl sulfoxide since it has been shown to increase the incidence of hepatocellular carcinoma in male $B6C3F_1$ mice. The concern is reinforced by the structural similarity of piperonyl sulfoxide with safrole, isosafrole, and dihydrosafrole which have been reported to be carcinogenic in rats and mice.

There is a **LOW** level of **concern over the extent of exposure**. Piperonyl sulfoxide is no longer used as an insecticide synergist in California or in the US. It was listed as an ingredient of several pesticide products; however registrations of all these products have now been canceled (CDPR, 1997).

References

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