FINAL PRIORITIZED CANDIDATE CHEMICALS UNDER CONSIDERATION FOR CARCINOGENICITY EVALUATION:

FIVE CHEMICALS WITHIN BATCH #3

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Final data summaries are available for the five remaining chemicals (lovastatin, methylphenidate and its hydrochloride (Ritalin), phenelzine and its acid salts, styrene, and tetrachlorvinphos) out of the 60 chemicals under consideration for carcinogenicity evaluation (Batch #3). Batch #3 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). One batch of 60 chemicals was selected in a second round pilot random selection from among 100 within Category I of the tracking database for which toxicity information had been entered into the toxicity field of the data entry sheet. On February 19, 1999, OEHHA announced the release of draft priority assignments and draft data summaries for 59 of 60 chemicals selected for prioritization with respect to their potential to cause cancer. The prioritization of one chemical, *bis*(4-chlorophenyl)sulfone, has been postponed pending the results of a bioassay expected in the next one to two years from the National Toxicology Program. The February 19, 1999, announcement initiated a 60-day public comment period, which included a public workshop held April 9, 1999. Fifty-four of the 59 priority assignments were finalized and announced in the *California Regulatory Notice Register* 99, No. 32-Z August 6, 1999. Review and careful consideration of the comments received on the remaining five chemicals has now been completed and the priority assignments have been finalized. The only final priority which differs from the draft assignment is for methylphenidate and its hydrochloride (Ritalin), for which the final priority is "not high" enough to merit placement on the Candidate List. Revisions have been made to the text of some data summaries as a result of comments.

Prioritized chemicals with a final priority of High Carcinogenicity Concern are assigned to the Candidate List, from which chemicals will be chosen for the preparation of hazard identification documents. All chemicals not assigned a final "high" level of carcinogenic concern are assigned to Category II. Action is not anticipated on Category II chemicals until all high priority chemicals on the Candidate List with known or potential exposure have been brought before the Committee. At that point, with Committee and public input, OEHHA will refine the existing process in order to determine which of the Category II prioritized chemicals should be brought forward for consideration by the CIC.

Exposure information is also assessed, according to the procedure outlined in the prioritization document of May 1997. This states that "A qualitative evaluation of the level of concern in terms of exposure will be expressed as 'high', 'medium', 'low', 'no identified concern' or 'inadequate data.'"

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

Name of Chemical	CAS No.	Level of Exposure Concern	Page
On Candidate List due to HIGH CARCINOGENICITY CONCERN			
Lovastatin	75330-75-5	High	3
Phenelzine and its acid salts	156-51-4	High	5
Styrene	100-42-5	High	8
Tetrachlorvinphos*	22248-79-9	High	15
Not on Candidate List			
Methylphenidate and its hydrochloride (Ritalin)	113-45-1	High	19

* This compound will not be forwarded to the CIC for consideration unless relevant new data unavailable to the State's qualified experts in April 1991 become available.

CARCINOGENICITY DATA SUMMARY: LOVASTATIN

Lovastatin (mevinolin; CAS No. 75330-75-5) is used as a drug to decrease elevated serum total and low-density lipoprotein cholesterol concentrations in the treatment of hyperchlolesterolemia. Lovastatin is marketed under the brand name Mevacor®. The recommended therapeutic dose range by the oral route is 0.2 to 1.6 mg/kg-day (MacDonald *et al.*, 1988).

Carcinogenicity Data available:

Epidemiological studies

A randomized, double-blind, placebo-controlled clinical trial of lovastatin was conducted at outpatient clinics in Texas (Pearson, 1998). This trial consisted of a total of 5608 men and 997 women with average total cholesterol and LDL levels and below-average HDL levels (as characterized by lipid percentiles for an age- and sex-matched cohort without cardiovascular disease from the National Health and Nutrition Examination Survey [NHANES] III). The treatment group received lovastatin (20-40 mg daily dose) or placebo. Both treated and placebo groups were placed on a low-saturated fat, low-cholesterol diet. After an average follow-up of 5.2 years, there were no clinically relevant differences in safety parameters, including cancer rates, between treatment and placebo groups. However, the short follow-up time limits any conclusions regarding the carcinogenicity of lovastatin in humans.

Animal bioassays

1. Mouse 92-week oral studies: As cited by HSDB, 1997; FDA, 1995; MacDonald *et al.*, 1988. Groups of 50 male and 50 female mice were treated orally with 0 (2 groups), 20, 100, or 500 mg/kg-day of lovastatin for 92 weeks. The incidences of hepatocellular carcinoma for control I, control II, 20, 100, or 500 mg/kg-day dose groups, respectively, were 2/50, 6/50, 5/50, 6/50 and 19/50 for males and 0/50, 0/50, 0/50, 0/50 and 7/50 for females. The incidence in the high dose-group relative to combined control incidence was statistically significant for both male and female mice ($p<0.001$). There was also a statistically significant increase in the incidence of pulmonary adenomas in high-dose female mice ($p<0.02$). In addition, the incidence rates of forestomach papillomas in the mid- and high-dose females were significantly higher than that of the controls ($p<0.02$).

2. Rat 2-year oral studies: HSDB, 1997; FDA, 1995; MacDonald *et al.*, 1988. Groups of 50 male and 50 female rats were treated with 0, 0, 5, 30, or 180 mg/kg-day of lovastatin for two years. The combined incidence of hepatocellular adenoma and carcinoma among male rats was not increased. Incidences of hepatocellular carcinoma observed among male rats were 0/50, 0/50, 2/50, 1/50, and 3/50 for control I, control II, 5, 30, and 180 mg/kg-day dose groups, respectively. The incidence in high-dose males was statistically significant ($p<0.05$) compared to the combined control incidence, but not compared to either control group alone. The increase in liver carcinomas in male rats was statistically significant by the trend test ($p=0.03$). The increased incidence of liver tumors was in the range normally seen in historical controls at the testing laboratory (MacDonald *et al*., 1988). No excess tumor incidences were observed among the female rats exposed to lovastatin.

Other relevant data

Lovastatin did not exhibit mutagenic potential in microbial systems (Ames test) with or without metabolic activation. It was negative in several *in vitro* mammalian cell systems (rat or mouse hepatocytes, Chinese hamster ovary cell, V-79 cell forward mutation study), and *in vivo* in mouse bone marrow chromosomal aberration studies (HSDB, 1997). Lovastatin is hydrolyzed to mevinolinic acid, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme essential in cholesterol biosynthesis. A product of HMG-CoA reductase is mevalonic acid which has been shown to be essential for DNA synthesis. There is some *in vitro* evidence that lovastatin can inhibit DNA synthesis during the S-phase of the cell life cycle through inhibition of HMG-CoA reductase; this inhibition appears to result from the depletion of mevalonic acid (HSDB, 1997). A number of other HMG-CoA reductase inhibitors such as pravastatin, simvastatin, and fluvastatin have also been shown to cause liver, lung or forestomach tumors in rodents.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as lovastatin has been shown to induce hepatocellular carcinoma in mice of both sexes. Further evidence of carcinogenicity includes statistically significant increases in the incidence of pulmonary adenomas in high-dose female mice, a dose-related increase in benign forestomach tumors in female mice,and the observation that other HMG-CoA reductase inhibitors also induce liver, lung, or forestomach tumors in rodents. A small increase in hepatocellular carcinomas was also observed in high-dose male rats.

There is a **HIGH** level of **concern over the extent of exposure** as lovastatin is widely prescribed to patients to control elevated cholesterol levels.

References

Food and Drug Administration (FDA, 1995). A request of carcinogenicity study for Lovastatin under the Freedom of Information Act to the Food and Drug Administration, Division of Federal-State Relations, Rockville, MD.

Hazardous Substances Data Bank (HSDB, 1997). National Library of Medicine. Bethesda, MD.

MacDonald JS, Gerson RJ, Kornbrust DJ, Kloss MW, Prahalada S, Berry PH, Alberts AW, Bokelman DL. (1988) Preclinical evaluation of lovastatin. *Am J Cardiol* **62**:16J-27J.

Pearson TA (1998). Lipid-lowering therapy in low-risk patients. *JAMA* **279**(20):1659-1661.

Physician's Desk Reference (PDR, 1997). Mevacor® Tablets. Medical Economics Company, Inc. Montvale, NJ. p. 1742.

CARCINOGENICITY DATA SUMMARY: PHENELZINE AND ITS ACID SALTS

Phenelzine (β-phenylethyl hydrazine, 2-phenylethyl hydrazine; CAS No. 156-51-4) is used to produce phenelzine sulfate (CAS No. 51-71-8), which is used solely as a prescription drug in the U.S. It acts as a monoamine oxidase inhibitor and is used in the treatment of certain types of depression and in certain phobic anxiety states. It is first given in a daily dose of 30-45 mg and then adjusted to 15-75 mg after the first two weeks (IARC, 1980). Only one company in the U.S. is believed to produce this drug. Phenelzine is used only in the preparation of the hydrochloride and sulfate salts (IARC, 1980). In 1978, U.S. imports of phenelzine sulfate through the principal U.S. customs districts amounted to 450 kg (IARC, 1980). Based upon limited evidence of carcinogenicity in experimental animals and inadequate evidence of carcinogenicity in humans, IARC (1980; 1987) has classified phenelzine sulfate as a Group 3 carcinogen. However, an evaluation using more recent versions of the inference guidelines used by IARC and other authorities might place more reliance on the observations of genetic toxicity, the structural analogy with several compounds known to cause cancer, and the case report of possible human carcinogenicity (see below). In addition, since the time of IARC's evaluation, additional genotoxicity data, including multiple observations of mutagenicity in bacteria, have become available.

Carcinogenicity Data available:

Epidemiological studies

A case report described a 64-year old woman treated with phenelzine for 6 years who developed angiosarcoma of the liver, multiple peritoneal angiosarcomas, and an osteolytic lesion in the humerus suggestive of metastatic disease (Daneshmend *et al.*, 1979; IARC, 1980). The patient had no history of exposure to other compounds suspected in the development of angiosarcomas (thorium dioxide, arsenic, vinyl chloride) and had been medicated only occasionally with diazepam.

Animal bioassays

1. Mouse lifetime drinking water studies: Toth and Shimizu, 1974; Toth, 1976; Toth and Nagel, 1976. Groups of 50 male and 50 female Swiss mice were given drinking water containing 0.015% phenelzine sulfate for a lifetime. The control groups consisted of 100 male and 100 female mice. In treated female mice, the incidence of lung adenomas or adenocarcinomas (combined) was significantly increased when compared to the incidence in controls (28/50 *vs.* 21/100: $p<0.001$), and the incidence of angiomas and angiosarcomas (combined) was also increased (22/50 *vs.* 5/100: $p<0.001$). In treated male mice, there was an increase in the incidence of lung adenomas or adenocarcinomas (combined) above the incidence in controls, but it was just short of being statistically significant (18/50 *vs.* 23/100: $p=0.07$).

Other relevant data

Male Sprague-Dawley rats fed diets containing phenelzine for 87 weeks and co-treated with subcutaneous injections of 1,2-dimethylhydrazine did not develop significantly increased intestinal adenocarcinomas over control animals, suggesting phenelzine is not co-carcinogenic (Gershbein and Rao, 1992).

In the absence of metabolic activation, phenelzine sulfate was mutagenic to *Salmonella typhimurium* strain TA100 (IARC, 1980; citing Shimizu *et al.*, 1978). Other studies also found that phenelzine sulfate was mutagenic to *Salmonella typhimurium* strain TA100, as well as to strain TA1535, but not to strains TA98, TA1537 and TA1538 (DeFlora, 1981; DeFlora *et al*., 1984); addition of S9 (Aroclor-induced rat liver enzymes) decreased the mutagenic response (DeFlora, 1981). Phenelzine sulfate has also been shown to inactivate *Bacillus subtilis* transforming DNA (IARC, 1980; citing Freese *et al.*, 1968) and to be reactive with DNA in the *pol* A^+/A^- test in *Escherichia coli* (IARC, 1980; citing Rosenkranz and Carr, 1971). Phenelzine was not found to damage DNA by the alkaline elution technique in liver and lung tissues of intraperitoneally treated Swiss mice (Parodi *et al.*, 1981). Phenelzine did not induce unscheduled DNA synthesis in rat or mouse hepatocytes, *in vitro* (Mori *et al*., 1988).

Phenelzine is structurally related to hydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, 1,2-diethylhydrazine and 1,2-diphenylhydrazine, all of which are listed under Proposition 65 as substances known to the State to cause cancer.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over phenelzine and its acid salts because phenelzine sulfate caused statistically significant increases in the incidences of malignant and benign lung tumors, and hemangiomas or hemangiosarcomas, in female mice. An increase in the incidence of malignant and benign lung tumors in male mice, although just short of statistical significance, adds to the concern, as do the observations of genotoxicity in bacterial test systems, and structural analogies with several known carcinogens.

There is a **HIGH** level of **concern over the extent of exposure** to phenelzine and its acid salts. Phenelzine sulfate is prescribed as an antidepressant drug. It is not usually the first choice for this condition, but is used in patients who have failed to respond to more commonly used antidepressant drugs. In 1998, this drug was prescribed to approximately 12,000 patients in the U.S. as a whole, and 1200 living in California. The daily dose prescribed to patients (e.g., 75 mg/day) may be equal to several percent of the dose that produced cancer in mice, and the drug may be administered to individuals on a long-term basis.

References

Daneshmend TK, Scott GL, Bradfield JWB (1979). Angiosarcoma of liver associated with phenelzine. *Br J Med* **i**:1679.

DeFlora S (1981). Study of 106 organic and inorganic compounds in Salmonella/microsome test. *Carcinogenesis* **2**:283-298.

Deflora S, Zanacchi P, Camoirano A, Bennicelli C, Badolati GS (1984). Genotoxicity and potency of 135 compounds in the Ames reversion test in a bacterial DNA-repair test. *Mutat Res* **133**:161-198.

Freese E, Sklarow S, Bautz Freese E (1968). DNA damage caused by antidepressant hydrazines and related drugs. *Mutat Res* **5**:343-8.

Gershbein LL, Rao KC (1992). Action of hydrazine drugs in tumor-free and 1,2-dimethylhydrazine-treated male rats. *Oncol Res* **4**(3):121-7.

International Agency for Research on Cancer (IARC, 1980*). IARC Monographs on the Carcinogenic Risk to Humans, Some Pharmaceutical Drugs.* Volume 24, pp. 175-84. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans; Overall evaluation of carcinogenicity: An updating of IARC monographs Volumes 1 to 42*. Supplement 7, pp. 312-313. IARC, Lyon, France.

Parodi S, De Flora S, Cavanna M, Pino A, Robbiano L, Bennicelli C, Brambilla G (1981). DNA-damaging activity *in vivo* and bacterial mutagenicity of sixteen hydrazine derivatives as related quantitatively to their carcinogenicity. *Cancer Res* **41**(4):1469-82.

Mori H, Sugie S, Yoshimi N, Iwata H, Nishikawa A, Matsukubo K, Shimizu H, Hirono I (1988). Genotoxicity of a variety of hydrazine derivatives in the hepatocyte primary culture/DNA repair test using rat and mouse hepatocytes *Jpn J Cancer Res (Gann)* **79**:204-211.

Rosenkranz HS, Carr HS (1971). Hydrazine antidepressants and isoniazid: potential carcinogens. *Lancet* **i**:1354-5.

Shimizu H, Hayashi K, Takemura N (1978). Relationships between the mutagenic and carcinogenic effects of hydrazine derivatives (Jpn). *Jpn J Hyg* **33**:474-85.

Toth B, Shimizu H (1974). 1-Carbamyl-2-phenylhydrazine tumorigenesis in Swiss mice. Morphology of lung adenomas. *J Natl Cancer Inst* **52**:241-51.

Toth B (1976). Tumorigenicity of β-phenylethylhydrazine sulfate in rats. *Cancer Res* **36**:917-21.

Toth B, Nagel D (1976). Tumorigenesis investigations with β-phenylethylhydrazine sulfate, phenylhydrazine hydrochloride, and ethylhydrazine hydrochloride in mice (Abstract no. 41). *Am J Pathol* **82**:40a-41a.

CARCINOGENICITY DATA SUMMARY: STYRENE

Styrene (CAS No. 100-42-5) is one of the most widely used chemicals in the U.S. It is estimated that 4 million tons were produced in 1992 in the U.S. (IARC, 1994). Styrene is used in the manufacture of polystyrene plastics, protective coatings, polyesters, synthetic rubber, copolymer resins with acrylonitrile and butadiene, and as a chemical intermediate. U.S. EPA is currently updating its evaluation on the toxicity and carcinogenicity of styrene. IARC (1994) reviewed styrene and concluded that the evidence of carcinogenicity in humans was inadequate and that the evidence in experimental animals was limited; however, IARC classified styrene as a Group 2B carcinogen: possibly carcinogenic to humans. In making the overall evaluation, IARC (1994) took into account a variety of supporting evidence, including evidence that styrene is metabolized to styrene-7,8-oxide, which binds covalently to DNA and is genotoxic in various *in vitro* and *in vivo* assays, evidence that styrene induces dose-related chromosomal damage in human whole blood cultures at low doses, and evidence that DNA and protein adducts and chromosomal damage have been detected in humans occupationally exposed to styrene. Since IARC's review, two additional epidemiological studies (Macaluso *et al*., 1996; Delzell *et al*., 1996) have been published and two animal bioassays commissioned by the Styrene Information and Research Council (SIRC, 1996 and 1998) were completed.

Carcinogenicity Data available:

Epidemiological studies

The epidemiological data were reviewed by IARC in 1994 and are summarized below. Most of the data were obtained from workers occupationally exposed to styrene in one of the three following industries: manufacture of styrene-butadiene rubber, manufacture and polymerization of styrene, and manufacture of reinforced plastic. In general, workers in the reinforced plastic industry were exposed to higher levels of styrene than those in the other two industries. In addition, workers in the reinforced plastic industry were less likely to have concurrent exposure to other agents, such as benzene and 1,3-butadiene, that are known to be associated with lymphatic and hematopoietic cancers. Of the epidemiological studies published, the data collected from the reinforced plastic industry are believed to be the most useful in evaluating the carcinogenicity of styrene in humans.

Manufacture of styrene-butadiene rubber

A number of earlier mortality studies of the rubber industry showed that working in the industry may be associated with elevated risk for cancers at various sites. However, workers in this industry are potentially exposed to many other chemicals besides styrene. In one study (McMichael *et al*., 1976, as cited in IARC, 1994) in which exposure to styrene-butadiene was specified, an association (relative risk [RR]=6.2; 99.9% confidence interval [CI], 4.1-13) between lymphatic and hematopoietic malignancies and employment in a workplace that used styrene-butadiene was found.

Meinhardt *et al*. (1982) studied two groups of workers in the styrene-butadiene rubber industry. An elevated rate of lymphatic and hematopoietic cancers was found in one group of workers (standard mortality rate [SMR] of 212, 95% CI, 97-402). The SMR for leukemias was 278 (95% CI, 90-648). The authors did not find any excess mortality or cancer deaths in the other group. Matanoski *et al*. (1990) studied a cohort of male workers in the styrene-butadiene industry and found no significant excess of cancer at any site for the total cohort. However, they observed an excess of all lymphatic and hematopoietic neoplasms in a subgroup of black production workers (SMR=5.1, 95% CI, 1.9-11; as reported in IARC, 1994). Three of the six malignancies were leukemias (SMR=6.6; 95% CI, 1.4-19; as reported in IARC, 1994). A case-control study of 59 cases of lymphohematopoietic cancer was conducted (Santos-Burgoa *et al*., 1992) within the cohort studied by Matanoski *et al*. (1990). They reported that exposure to 1,3-butadiene was significantly associated with lymphatic and hematopoietic cancers, whereas exposure to styrene was not.

In a recent study, Macaluso *et al*. (1996) studied leukemia mortality among 16,610 subjects employed at 6 styrene-butadiene rubber manufacturing plants. Based on work histories, exposure levels for styrene, 1,3-butadiene, and benzene of the workers were estimated. The authors reported that there was no evidence to suggest that exposure to styrene was associated with increased incidence of leukemia. However, they did find that exposure to 1,3-butadiene was associated with a dose-related increase in the occurrence of leukemia. Delzell *et al*. (1996) studied a group of workers employed for at least a year in the styrene-butadiene rubber industry. They estimated that approximately

75% of the subjects were exposed to 1,3-butadiene and 83% were exposed to styrene. More leukemia deaths were observed than expected within the cohort (SMR=131; 95% CI, 97-174). Excess leukemia deaths were found primarily among workers with a long work history and a long follow-up period, as well as among subjects who had worked in areas with the potential for relatively high exposure to 1,3-butadiene or styrene.

Manufacture and polymerization of styrene

Ott *et al*. (1980, as referenced in IARC, 1994) studied mortality rates and tumor incidence rates among workers employed by the styrene manufacture and polymerization industry between 1937 and 1960. They found mortality and all neoplasms were lower than expected. Bond *et al*. (1992, as referenced in IARC, 1994) updated the study, adding a further 11 years of observations. They also found mortality from all causes and all neoplasms was lower than expected (overall SMR=76; 95% CI, 70-82; cancer SMR=81; 95% CI, 69-95), but cancers of the hematopoietic system were higher than expected (SMR=144; 95% CI, 95-208). Besides styrene, the workers were also potentially exposed to other chemicals such as benzene, acrylonitrile, 1,3-butadiene, ethylbenzene, dyes, and pigments. Hodgson and Jones (1985) studied a group of workers who had worked for at least a year in the production, polymerization, and processing of styrene between 1945 and 1978. No measurements of exposure were provided in the study, but many other chemicals were present in the working environment. The authors found a significant increase in lymphatic and hematopoietic cancer among the exposed workers (standardized incidence ratio=250; CI, 67-640; as reported in IARC, 1994).

Manufacture of reinforced plastic

Okun *et al*. (1985, as referenced in IARC, 1994) studied 5,021 workers who had worked in two reinforced plastic boat-building facilities between 1959 and 1978. No elevated mortality or cancer incidence rates were observed. Coggon *et al*. (1987, as referenced in IARC, 1994) studied a cohort of workers in the reinforced plastics industry in Britain and reported no increase in either mortality rates or hematopoietic cancer. However, they did report a slight increase in cancers of the respiratory system among workers exposed to styrene (SMR=126, 95% CI, 94-166). Kogevinas *et al*. (1994, as referenced in IARC, 1994) followed the same cohort and reported that by 1990 the excess of lung cancer was less marked, with a SMR of 106 (95% CI, 84-132). Kogevinas *et al*. also studied a larger cohort of workers in Europe and found no excess in mortality from all causes (SMR=96; 95% CI, 92-100) or from all neoplasms (SMR, 91; 95% CI, 83-98). The mortality rate in exposed workers for cancers of the lymphatic and hematopoietic tissues was not elevated (SMR=96; 95% CI, 71-127) and was not associated with length of employment. For two cohorts in Britain and Denmark, however, there were moderate increases in mortality from lymphatic and hematopoietic cancer (Britain: SMR=121; 95% CI, 25-355; Denmark: SMR=122; 95% CI, 78-181). Wong *et al*. (1994, as referenced in IARC, 1994) studied a cohort of workers employed by reinforced-plastics plants in the U.S. between 1948 and 1977. They reported significantly increased rates of tumors at various sites: oesophagus (SMR=198; 95% CI, 105-322), respiratory system (SMR=141; 95% CI, 120-164), cervix uteri (SMR, 284; 95% CI, 136-521), and other female genital organs (SMR=202; 95% CI, 107-345). These increases were mainly among short-term workers with low cumulative exposures. Although Wong *et al*. (1994) did not observe any excess in lymphatic and hematopoietic cancers (SMR=82; 95% CI, 56-117) for all styrene exposed workers, for workers in jobs associated with high styrene exposure, the SMR for lymphatic and hematopoietic cancers was 141, and for the group with highest cumulative exposure and latency greater than 20 years, the SMR was 134. Kolstad *et al*. (1994, as referenced in IARC, 1994) studied a group of male Danish workers employed by industries that had ever produced reinforced plastics, and found elevated rates of leukemia and lymphomas among some groups of workers. In workers who were first employed more than 10 years before, the standard incidence rate (SIR) for leukemia was 157 (95% CI, 107-222; as reported in IARC, 1994); the excess was due to cases in workers who were employed for less than one year. For those employed in 1964-70, the SIR for leukemia more than 10 years after first short-term employment was 2.3 (95% CI, 1.4-3.6); however, for workers with more than one year employment, the corresponding SIR was 1.0 (95% CI, 0.52-1.7). For workers with less than 10 years since first employment, the only significant increase was for lymphomas (SIR=1.7; 95% CI, 1.0-2.5), with similar increases for short- and long-term employees.

Animal bioassays

1. Mouse long-term inhalation studies: SIRC, 1998. (Data not published in peer-reviewed literature). Groups of 70 male and 70 female CD-1 mice were exposed to styrene at 0, 20, 40, 80, or 160 ppm, 6 hours a day, 5 days a week. Ten mice of each sex and dose group were sacrificed following 52 and 78 weeks of exposure. Due to increased mortality in female control mice, terminal sacrifice of females took place during week 98. Male mice

were sacrificed after 104 weeks of exposure. Body weight gain was reduced in male and female mice exposed to 80 or 160 ppm. Increased incidences of pulmonary bronchiolar-alveolar adenomas were observed in females [6/50, 16/50 ($p<0.05$), 16/50 ($p<0.05$), 11/50, and 24/50 ($p<0.01$) for the control, 20, 40, 80, and 160 ppm groups, respectively] and in males [15/50, 21/50, 35/50 ($p<0.01$), 30/50 ($p<0.01$), and 33/50 ($p<0.01$)]. An increased incidence of pulmonary bronchiolar-alveolar carcinomas was observed in the high-dose females [0/50, 0/50, 2/50, 0/50, and 7/50 ($p<0.01$)]. The incidence of this tumor was not significantly increased in the males [4/50, 5/50, 3/50, 6/50, and 7/50].

2. Mouse long-term gavage studies: NCI, 1979a. Groups of 50 male and 50 female $B6C3F_1$ mice were treated with 150 or 300 mg/kg body weight styrene by gavage in corn oil on five days per week for 78 weeks, followed by an observation period of 13 weeks. Control groups of 20 male and female mice received corn oil only. Body weights of treated females were slightly reduced, and survival was slightly reduced in high-dose males (20/20, 46/50, 39/50 for control, low- and high-dose groups, respectively) and females (18/20, 40/50, 38/50). There was a significant positive association between dosage and the incidence of combined adenomas and carcinomas of the lung in male mice [0/20, 6/44 (13.6%), and 9/43 (20.9%)]. The incidence of these tumors in the high-dose group was also statistically significantly increased as compared to the controls ($p=0.024$). However, NCI noted that the incidence of these tumors among untreated historical controls at the laboratory was 32/271 (12%) ($p=0.08$ when the incidence of the high-dose group is compared with that of the historical controls). No statistically significant differences were observed between tumor incidence at any sites in the treated female mice and controls. NCI reported that there was suggestive evidence to indicate administration of styrene is associated with an increased incidence of a combination of adenomas and carcinomas of the lung in male $B6C3F_1$ mice. However, NCI concluded that this bioassay did not provide convincing evidence for the carcinogenicity of styrene in $B6C3F_1$ mice of either sex. In their review of the bioassay, IARC (1994) noted that there was a significant increasing trend in the incidence of hepatocellular adenoma in female mice: 0/20, 1/44, and 5/43.

3. Mouse long-term gavage studies: NCI, 1979b. Groups of 50 male and 50 female $B6C3F_1$ mice were treated with 203 or 406 mg/kg body weight styrene in a mixture (solution of 70% styrene and 30% ß-nitrostyrene) in corn oil by gavage, three days per week for 78 weeks. This was followed by an additional 14-week observation period. Control groups of 20 male and female mice received corn oil only. Body weights of high-dose female mice were slightly reduced. Survival among males was 18/20 (control), 43/50 (low-dose), and 33/50 (high-dose), and among the female was 17/20, 47/50, and 38/50. The combined incidence of adenoma and carcinoma of the lung in male mice was 0/20 (control), 11/44 (low-dose; $p=0.016$), and 2/43 (high-dose) (IARC, 1994). However, the trend test and the high dose to control comparison were not statistically significant. No other tests for tumors at any site in either male or female mice were statistically significant. NCI concluded that there was no convincing evidence for the carcinogenicity of a solution of ß-nitrostyrene and styrene in $B6C3F_1$ mice of either sex.

4. Mouse single prenatal exposure and long-term gavage studies: Ponomarkov and Tomatis, 1978, as cited in IARC, 1994. A group of 29 pregnant O20 mice received a single dose of 1,350 mg/kg styrene in olive oil by gavage on day 17 of gestation. A control group of 9 pregnant mice received olive oil alone. Groups of 45 male and 39 female progeny from the dams that received styrene were administered 1,350 mg/kg styrene by gavage once a week from weaning until 16 weeks of age. Control groups of 20 males and 22 females with no prenatal exposure received olive oil alone. Administration of styrene was stopped at 16 weeks because of high mortality related to treatment. The experiment was terminated at 120 weeks. In the progeny that received weekly administration of styrene, the combined incidence of lung adenomas and carcinomas was significantly ($p<0.01$) increased over that in the vehicle controls: males, 8/19 controls versus 20/23 treated, and females, 14/21 controls versus 32/32 treated. There was no treatment-related difference in the incidence of tumors at other sites in the progeny (IARC, 1994).

5. Mouse single prenatal exposure and long-term gavage studies: Ponomarkov and Tomatis, 1978, as cited in IARC, 1994. A group of 15 pregnant C57B1 mice received a single administration of 300 mg/kg in olive oil by gavage on day 17 of gestation. A control group of 5 pregnant mice received olive oil only. Groups of 27 male and 27 female progeny from the dams that received styrene were administered 300 mg/kg styrene in olive oil by gavage once a week from weaning up to 120 weeks. Control groups of 12 male and 13 female mice received

olive oil alone. There were no treatment-related effects on body weight or survival. There were no treatment-related difference in the incidences of tumors at any site in the progeny (IARC, 1994).

6. Mouse intraperitoneal injection study: Brunnemann *et al*., 1992, as cited in IARC, 1994. A group of 25 female A/J mice received intraperitoneal injections of 20 µmol styrene in olive oil three times a week for a total of 20 injections. A vehicle control group of 25 mice received olive oil alone. The study was terminated 20 weeks after the last injection. There was no treatment-related increase in the incidence of lung tumors (3/25 versus 1/25 in controls).

7. Rat long-term inhalation studies: SIRC, 1996; Cruzan *et al*., 1998. Groups of 70 male and 70 female Sprague-Dawley rats were exposed to styrene at 0, 50, 200, 500, or 1000 ppm for 104 weeks, 6 hours a day, 5 days a week. Ten rats of each sex and dose group were sacrificed after 52 weeks of exposure. Body weight gain was reduced in groups exposed to 500 or 1000 ppm, particularly during the first year of the study. Survival by male rats was not affected by styrene exposure at levels up to 1000 ppm for 104 weeks. There was increased survival of females exposed to styrene at 500 or 1000 ppm. In rats killed at 52 weeks or later, there was a significant dose-related increase of interstitial cell tumors of the testes in male rats (2/60, 2/60, 2/60, 4/54, and 6/52 for the control, 50 ppm, 200 ppm, 500 ppm, and 1000 ppm groups, respectively). However, pairwise comparisons of the high-dose groups against the control group were not statistically significant. Comparison with historical control data showed that the incidences of interstitial cell tumors in the high-dose groups were within the background range.

8. Rat 52-week inhalation studies: Maltoni *et al*., 1982 and Conti *et al*. 1988, as referenced in IARC, 1994. Groups of 30 male and 30 female Sprague-Dawley rats were exposed by inhalation to 25, 50, 100, 200 or 300 ppm styrene for 4 hour per day, five days a week, for 52 weeks. The control groups were comprised of 60 male and 60 female rats. The study was terminated when the last animal died. No treatment-related effects on body weight or survival were reported. There was a significant ($p<0.01$, Cochran-Armitage trend test) correlation between dose and incidence of malignant mammary tumors in female rats: 6/60 controls, 6/30 at 25 ppm, 4/30 at 50 ppm, 9/30 at 100 ppm, 12/30 at 200 ppm and 9/30 at 300 ppm. The incidence rates of the three highest dose groups were also significantly higher than that of the controls ($p<0.05$, Fisher exact test for the 100, 200, and 300 ppm groups). The combined incidence of benign and malignant mammary tumors was also greater in treated female rats than in controls (34/60, 24/30, 21/30, 23/30, and 25/30).

9. Rat long-term drinking water studies: Beliles *et al*., 1985. Groups of 50 male and 70 female Sprague-Dawley rats were treated with 125 or 250 ppm styrene (nominal concentrations) daily in the drinking water for 104 weeks. Control groups of 76 male and 104 female rats received drinking water without styrene. At 52 weeks, 10 rats per sex and group were removed and killed. There was a significant reduction in water consumption among treated male and female rats and a significant reduction in body weight among high-dose females. There was no treatment-related effect on survival and no evidence of carcinogenicity. IARC (1994) reviewed the study results and noted the low level of exposure.

10. Rat long-term gavage studies: NCI, 1979a. Groups of 50 male and 50 female Fischer 344/N rats were treated with 1,000 or 2,000 mg/kg body weight styrene by gavage in corn oil five days per week for 78 weeks. Because of high mortality in the high-dose groups by week 23, additional groups of 50 male and 50 female rats administered 500 mg/kg styrene for 103 weeks were included in the study. Control groups of 20 male and 20 female rats received corn oil only. Treatment was followed by an observation period of 27 weeks for high- and mid-dose rats, and 1 week for low-dose rats. Survival was poor in both high-dose males and females. There was no treatment-related increase in the incidence of any type of tumor in male or female rats. NCI concluded that there was no convincing evidence for the carcinogenicity of styrene in Fischer 344 rats of either sex.

11. Rat long-term gavage studies: NCI, 1979b. Groups of 50 male and 50 female Fischer 344/N rats were treated with 350 and 700 mg/kg body weight (males) or 175 and 350 mg/kg bw (females) styrene in a mixture (solution of 70% styrene and 30% ß-nitrostyrene) in corn oil by gavage. The chemical was administered three days per week for 78 weeks, followed by an additional observation period of 29 weeks. Control groups of 20 male and 20 female rats received corn oil only. The body weights of male rats were slightly reduced. There was no effect on survival in male (16/20, 34/50, 31/50) or female (12/20, 33/50, 31/50) rats (IARC, 1994). There was

no treatment-related increase in the incidence of any type of tumor in male or female rats. NCI concluded that there was no convincing evidence for the carcinogenicity of a solution of β-nitrostyrene and styrene in Fischer 344/N rats of either sex.

12. Rat 52-week gavage studies: Conti *et al*., 1988, as referenced in IARC, 1994. Groups of 40 male and 40 female Sprague-Dawley rats were treated with 0, 50 or 250 mg/kg body weight styrene in olive oil by gavage on four to five days per week for 52 weeks. The study was terminated when the last animal died. There was no treatment-related effect on body weight; survival of female rats receiving the high dose was reduced. There was no treatment-related increase in the incidence of any type of tumor.

13. Rat single prenatal exposure and long-term gavage studies: Ponomarkov and Tomatis, 1978, as referenced in IARC, 1994. A group of 21 pregnant BDIV rats received a single administration of 1,350 mg/kg in olive oil by gavage on day 17 of gestation. A control group of 10 pregnant rats received olive oil only. Groups of 73 male and 71 female progeny of dams that received styrene were administered 500 mg/kg styrene in olive oil by gavage weekly from weaning up to 120 weeks. Control groups of 36 male and 39 female rats received olive oil alone. The experiment was terminated at 120 weeks. There were no treatment-related effects on body weight or survival. At the time of observation of the first tumor, 32 controls and 54 treated male progeny and 35 control and 68 treated female progeny were still alive. Stomach tumors occurred in three female rats (adenoma, fibrosarcoma, carcinosarcoma) administered styrene and in one female rat (fibrosarcoma) in the control group. There was no significant treatment-related increase in tumor incidence at any site (IARC, 1994).

14. Rat intraperitoneal injection studies: Conti *et al*., 1988 as referenced in IARC, 1994. Groups of 40 male and 40 female Sprague-Dawley rats were administered 4 intraperitoneal injections of 50 mg/animal in olive oil at two-month intervals. Control groups received injections of olive oil alone. The study was terminated when the last animal died. There was no treatment-related increase in the incidence of benign and/or malignant tumors.

Other Relevant Data:

Styrene is readily absorbed via inhalation and is mainly metabolized in the liver to styrene-7,8-oxide, a compound known to the State of California to cause cancer. Though most of the styrene-7,8-oxide produced in humans is detoxified by hydrolysis or conjugation with glutathione, styrene-7,8-oxide has been detected in blood samples of workers exposed to styrene in air (Korn *et al*., 1994; IARC, 1994). In *in vivo* and *in vitro* studies, it has been shown that styrene metabolites bind covalently to DNA, hemoglobin, and albumin (IARC, 1994). Using ^{32}P-post-labelling techniques, Horvath *et al.* (1994) found a significant linear relationship between styrene exposure and styrene-7,8-oxide-DNA adduct levels in the mononuclear cells of styrene workers. A dose-related increase of styrene-7,8-oxide-hemoglobin adduct levels was detected in a group of workers from a fiberglass reinforced plastic factory (Brenner *et al*., 1991 as referenced in IARC, 1994). Many cytogenetic studies had been conducted on workers occupationally exposed to styrene, with mixed results. Bonassi *et al.* (1996) performed a meta-analysis of 25 biomonitoring studies of occupational exposure to styrene. They found a significant increase of chromosomal aberrations was apparent in studies performed on workers with high levels of exposure to styrene, while inconclusive data were obtained for sister chromatid exchanges (SCEs) and micronuclei (MN) tests. Yager *et al.* (1993) found elevated levels of SCEs in blood lymphocytes of workers exposed to styrene. Recently, Yeowell-O'Connell *et al.* (1996) and Rappaport *et al*. (1996) reported that they detected styrene-7,8-oxide in the work room air of a plant that makes styrene reinforced plastics. It is possible that some of the cytogenetic effects observed in workers of the styrene reinforced plastic industry were related to direct exposure to styrene-7,8-oxide.

Most studies reported negative results for bacterial mutagenicity of styrene with or without exogenous metabolic activation (IARC, 1994). A few positive responses were reported in strains TA1535 and TA100 of *Salmonella typhimurium* in the presence of exogenous metabolic activation. Styrene was reported to be negative in inducing both forward gene mutation and mitotic gene conversion in the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* in the presence of mouse liver microsomes (Barale, 1991). Cytogenetic studies on *Allium meristematic* root tips indicated that styrene was able to induce chromosome breaks, anaphase bridges and micronuclei. Styrene was shown to be weakly positive in inducing X-linked recessive lethal mutations in *Drosphila melanogaster* (Barale, 1991). Styrene has been shown to induce SCEs, chromosomal aberrations, and MN in human lymphocytes and other mammalian cells *in vitro* (IARC, 1994). Kligerman *et al.* (1993, as referenced in IARC, 1994) reported elevated levels of SCEs in rats and mice exposed to styrene via inhalation, although most *in vivo*

studies in rodents did not find significant increases in clastogenic effects (SCE, MN, chromosomal abberrations) (IARC, 1994; Scott and Preston, 1994).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over styrene, since administration of the chemical has been shown to induce malignant lung tumors in female mice exposed via inhalation, and a dose-related increase in malignant and benign lung tumors in male mice exposed by gavage. In a study where pregnant mice and their offspring were exposed to styrene by gavage, elevated incidences of malignant and benign lung tumors were observed in the male and female offspring. In the rat, inhalation exposure to styrene resulted in a dose-related increase of testicular tumors in males in one series of studies, while in another series of inhalation studies conducted in the same rat strain, a significant increase in malignant mammary tumors was observed in females. Further evidence of carcinogenicity includes observations that styrene is clastogenic in numerous *in vitro* and *in vivo* tests in human and mammalian cells and mutagenic in some bacterial test systems. The level of concern is further reinforced by the detection of styrene-7,8-oxide-DNA adducts and styrene-7,8-oxide-hemoglobin adducts in blood samples of workers occupationally exposed to styrene. There is also human data suggesting a possible link between styrene exposure and hematopoietic cancer.

There is a **HIGH** level of **concern over the extent of exposure**. NIOSH (1983) estimated that 301,013 workers in 21,697 manufacturing facilities were potentially exposed to styrene at work. Occupational exposure to styrene is highest in the glass-reinforced plastic industry. Eight-hour time-weighted average air concentrations ranging from 20 to 100 ppm have been reported for this industry (IARC, 1994). Levels of 26 to 71 ppb styrene have been reported in indoor air in high-rise apartments (HSDB, 1995). The statewide mean ambient air concentration measured in California was 0.07 ppb in 1995; the highest sample measured contained 0.7 ppb styrene (ARB, 1998). Besides contaminated air, the general population is also exposed to styrene through the use of consumer products that contain styrene, by ingestion of food packaged in polystyrene, by ingestion of contaminated drinking water, and by inhalation of tobacco smoke (HSDB, 1995). Styrene has also been detected in human breast milk samples collected from four U.S. cities (HSDB, 1995).

References

Air Resources Board (ARB, 1998). Air Resources Board Homepage. Statewide styrene summary data, 1990-1995. Available from http://arbis.arb.ca.gov/aqd/styr/ststate.htm.

Barale R (1991). The genetic toxicology of styrene and styrene oxide. *Mutat Res* **257**:107-126.

Beliles RP, Butala JH, Stack CR, Makris S (1985). Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. *Fund Appl Toxicol* **5**:855-868.

Bonassi S, Montanaro F, Ceppi M, Abbondandolo A (1996). Is human exposure to styrene a cause of cytogenetic damage? A re-analysis of the available evidence. *Biomarkers* **1**:217-225.

Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Hardy CJ, Coombs DW, Mullins PA, Brown WR (1998). Chronic toxicity/oncogenicity study of stryene in CD rats by inhalation exposure for 104 weeks. *Toxicol Sci* **46**:266-281.

Delzell E, Sathiakumar N, Hovinga M, Macaluso M, Julian J, Larson R, Cole P, Muir DCF (1996). A follow-up study of synthetic rubber workers. *Toxicol* **113**:182-189.

Hodgson JT, Jones RF (1985). Mortality of styrene production, polymerization and processing workers at a site in Northwest England. *Scand J Work Environ Health* **11**:347-352.

Horvath E, Pongracz K, Rappaport S, Bodell WJ (1994). 32P-post-labelling detection of DNA adducts in mononuclear cells of workers occupationally exposed to styrene. *Carcinogenesis* **15**(7):1309-1315.

Hazardous Substances Data Bank (HSDB, 1995). National Library of Medicine. Bethesda, MD.

International Agency for Research on Cancer (IARC, 1994). *IARC monographs on the evaluation of carcinogenic risks to humans; Some industrial chemicals.* Volume 60, pp. 233-320. IARC, Lyon, France.

Korn M, Gfrörer W, Filser JG, Kessler W (1994). Styrene-7,8-oxide in blood of workers exposed to styrene. *Arch Toxicol* **68**:524-527.

Macaluso M, Larson R, Delzell E, Sathiakumar N, Hovinga M, Julian J, Muir D, Cole P (1996). Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. *Toxicol* **113**:190-202.

Matanoski GM, Santos-Burgoa C, Schwartz L (1990). Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982). *Environ Health Perspect* **86**:107-117.

Meinhardt TJ, Lemen RA, Crandall MS, Young RJ (1982). Environmental epidemiologic investigation of the styrene-butadiene rubber industry. Mortality patterns with discussion of the hematopoietic and lymphatic malignancies. *Scand J Work Environ Health* **8**:250-259.

National Cancer Institute (NCI, 1979a). Bioassay of styrene for possible carcinogenicity. National Cancer Institute, Carcinogenesis Technical Report No. 185. US Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI, 1979b). Bioassay of a solution of ß-nitrostyrene and styrene for possible carcinogenicity. National Cancer Institute, Carcinogenesis Technical Report No. 170. US Department of Health, Education, and Welfare, Public Health Service, National Institute of Health, Bethesda, MD.

National Institute for Occupational Safety and Health (NIOSH, 1983). Criteria for a recommended standard. Occupational exposure to styrene. NIOSH Publication No. 83-119, National Institute for Occupational Safety and Health, Cincinnati, OH.

Rappaport SM, Yeowell-O'Connell K, Bodell W, Yager JW, Symanski E (1996). An investigation of multiple biomarkers among workers exposed to styrene and styrene-7,8-oxide. *Cancer Res* **56**:5410-5416.

Santos-Burgoa C, Matanoski GM, Zeger S, Schwartz L (1992). Lymphohematopoietic cancer in sytrene-butadiene polymerization workers. *Am J Epidemiol* **136**:843-854.

Scott D, Preston RJ (1994). A re-evaluation of the cytogenetic effects of styrene. *Mutat Res* **318**:175-203.

Styrene Information and Research Council (SIRC, 1996). Styrene - 104-week repeat dose inhalation combined toxicity/carcinogenicity study in rats. Volume 1. Huntingdon Life Sciences.

Styrene Information and Research Council (SIRC, 1998). Styrene - 104-week repeat dose inhalation combined toxicity/carcinogenicity study in mice. Volume 1. Huntingdon Life Sciences.

Yager JW, Paradisin WM, Rappaport SM (1993). Sister-chromatid exchanges in lymphocytes are increased in relation to longitudinally measured occupational exposure to low concentrations of styrene. *Mutat Res* **319**:155-165.

Yeowell-O'Connell K, Jin Z, Rappaport SM (1996). Determination of albumin and hemoglobin adducts in workers exposed to styrene and styrene oxide. *Cancer Epidemiol Biomarkers Prevention* **5**:205-215.

CARCINOGENICITY DATA SUMMARY: TETRACHLORVINPHOS

Tetrachlorvinphos (2,4,5-trichloro-alpha-(chloromethylene) benzyl phosphate ester, 2-chloro-1-(2,4,5-trichlorophenyl)-vinyl dimethyl phosphate, Dietreen™, Gardona™, Rabon™, Stirohpos™; CAS No. 22248-79-9 [*cis*-isomer] or 961-11-5 [mixed isomers]) is an organophosphorus compound and is actively registered for 85 products in California for use as an insecticide on livestock and pets (and formerly on food crops), and as a feed additive for beef and dairy cattle, horses, and swine (CDPR, 1999). The 1997 Summary of Pesticide Use Report Data indicates that over 6000 lbs of tetrachlorvinphos were applied in California in 1997 on over 350 acres, with approximately 80% of this use on animal husbandry premises (CDPR, 1999). Approximately 45,000 kg (99,000 lbs) of tetrachlorvinphos were used in the U.S. in 1978 (IARC, 1983). Tolerances in the U.S. for residues of tetrachlorvinphos in or on raw agricultural commodities are 0.1-110 mg/kg; for a variety of 18 fodders the tolerance is 110 mg/kg, for fruits, grains, livestock and poultry fats, and eggs the tolerance is 0.1 mg/kg (IARC, 1983). IARC classified tetrachlorvinphos as a Group 3 carcinogen based on insufficient human data and limited evidence from animal studies (IARC, 1983). U.S. EPA classified tetrachlorvinphos as a Group C carcinogen (U.S. EPA, 1997). The compound was considered by the Proposition 65 Scientific Advisory Panel and rejected for listing on April 26, 1991. Some additional data not considered by the Panel demonstrating positive genotoxic potential of the compound in several test systems and species have been published (Amer and Aly, 1992; Kurinnyi, 1975; Vallini *et al.*, 1983).

Carcinogenicity Data available:

Epidemiological studies

No human carcinogenicity studies were reported by IARC (1983) or found in a search of the more recent scientific literature by OEHHA.

Animal bioassays

1. Rat long-term dietary studies: NCI, 1978. Groups of 50 male and 50 female Osborne-Mendel rats were administered tetrachlorvinphos (technical grade, 98% purity) at one of two doses for 80 weeks, then observed for 31 additional weeks. Time-weighted average doses were either 4250 or 8500 ppm. Matched controls consisted of 10 untreated rats of each sex; statistical comparisons were based on both matched controls and pooled controls of 55 male and 55 female rats. The incidence of C-cell adenoma of the thyroid in female rats showed a significant dose-related trend (pooled controls, 1/46; low-dose, 2/50; high-dose, 7/46 (p=0.013)). The incidence of tumors found in the high-dose group was significantly higher than that of the pooled controls (p=0.027). The incidence of C-cell hyperplasia of the thyroid in both low-dose (7/50) and high-dose (16/46) females was significantly higher compared with that in pooled controls (1/50). In addition, cortical adenoma of the adrenal gland also showed a significant dose-related trend in the females (pooled controls, 0/50; low-dose, 2/49; high-dose, 5/50 (p=0.017)). The incidence of adenomas in the high-dose females was significantly higher than that of the pooled controls (p=0.022). Hemangioma of the spleen occurred in male rats at a significantly higher incidence in the low-dose group than in the pooled controls (controls, 0/52; low-dose, 4/48); however, neither the incidence in the high-dose group (0/47) nor the test for a dose-related trend was statistically significant. NCI concluded that administration of technical grade tetrachlorvinphos to Osborne-Mendel rats was associated with proliferative lesions of the C cells of the thyroid and cortical adenomas of the adrenal in females. IARC reviewed the bioassay and noted the short duration of treatment (80 weeks) and period of observation (31 weeks) prior to termination of the study (IARC, 1983).

2. Rat long-term dietary studies: Walker *et al.*, 1972 as cited in IARC, 1983. Groups of 25 male and 25 female Porton rats were fed 5, 25, 125, or 2000 ppm (mg/kg) tetrachlorvinphos (95% pure, E-isomer) in the diet for 24 months. Groups of 45 males and 45 females served as controls. There were no significant differences in tumor incidence between treated and control rats. However, this study was limited by the relatively low doses administered and the small numbers of animals per dose group.

3. Rat long-term dietary studies: Inveresk Research International Ltd. (1993). Groups of 50 Sprague-Dawley rats of each sex were administered tetrachlorvinphos (technical grade, 99% pure), incorporated in the diet at 100, 1000 or 2000 ppm, for 104 weeks. Control groups received plain diet. Average dose rates for the treated males

were 5.4, 46 and 93 mg tetrachlorvinphos/kg-day, and for the treated females 6.2, 65 and 129 mg tetrachlorvinphos/kg-day. No significant effects of tetrachlorvinphos treatment were observed on mortality in either sex, or on body weight increase in the males. In females, the intermediate- and high-dose groups showed reduced weight gain at 104 weeks, but these effects (12% and 9% respectively) were fairly small and did not show a clear dose-response. Larger effects (14% and 17% in the intermediate and high dose groups) were seen in the females only at 52 weeks. No significant increase in tumor incidence relative to controls was noted in any treated group. Non-neoplastic findings included diffuse lipidosis in the adrenals in high-dose rats of both sexes, and female rats in the intermediate dose group. In the liver, minimal to moderate hypertrophy of centrilobular hepatocytes was noted in high-dose rats of both sexes, and female rats in the intermediate dose group. Occasional multinucleate hepatocytes were noted in some females at 52 weeks, and in high-dose males some lipid droplets at 52 weeks, and degenerative changes at 104 weeks, were noted. Moderate decreases (up to 68%) in plasma cholinesterase were noted at all time points and in both sexes at the intermediate and high dose levels. Smaller decreases in red blood cell cholinesterase were noted in the intermediate and high dose group females (29% and 36%, respectively) at weeks 77/78 only.

4. Mouse long-term dietary dietary studies: NCI, 1978. Groups of 50 male and 50 female $B6C3F_1$ mice were administered tetrachlorvinphos (technical grade, 98% purity) at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 12 additional weeks. Matched controls consisted of 10 untreated mice of each sex; statistical comparisons were based on both matched controls and pooled controls of 50 male and 50 female mice. The incidence of hepatocellular carcinoma in treated male mice was significantly higher than the controls (pooled controls, 5/49; low-dose, 36/50; high-dose, 40/50). The dose-related trend was also statistically significant ($p<0.001$). In females, the incidence of hepatocellular carcinoma was not significant (0/9, 2/48, 5/49, and 2/47 for the matched controls, pooled controls, low-dose, and high-dose groups, respectively); however, the combined incidence of hepatocellular carcinomas and neoplastic nodules in the low- and high-dose groups (19/49 and 11/47, respectively) was significantly higher than that of the pooled controls (3/48). NCI concluded that in female mice the incidence of neoplastic nodules of the liver was associated with treatment, and in male mice tetrachlorvinphos was carcinogenic, causing hepatocellular carcinoma of the liver.

5. Mouse long-term dietary dietary studies: Parker *et al*., 1985. Groups of 80 male and 80 female $B6C3F_1$ mice were administered tetrachlorvinphos (98.7% purity) in the diet for two years at 17.5, 64, 320, 1600, 8000 or 16,000 ppm. Another group of 80 male and 80 female mice was fed 16,000 ppm tetrachlorvinphos of a different batch. There were 160 male and 160 female mice in the control groups. Ten treated and 20 control mice/sex/group were killed at 6, 12, and 18 months. Animals treated with 8000 and 16,000 ppm tetrachlorvinphos showed severely depressed weight gain, suggesting that the maximum tolerated dose might have been exceeded. Different results were reported by the two pathologists that examined the slides. According to the study pathologist, there was a significant ($p<0.05$) increase of hepatocellular carcinoma in one group of male mice exposed to 16,000 ppm (24/99 and 35/46 for controls and high-dose animals, respectively). However, according to the consultant pathologist, the incidences of hepatocellular carcinoma in these two groups were not statistically different (14/99 and 6/46 for controls and high-dose animals, respectively). The incidence of renal tubular adenoma and carcinoma was significantly increased ($p<0.05$) in male mice fed 16,000 ppm tetrachlorvinphos (1/99, 11/50 and 12/46 for controls, and the two high-dose groups, respectively). However, the two pathologists differed in their opinions on the malignancy of these tumors. The study pathologist considered the majority of these tumors to be carcinomas, while the consultant pathologist diagnosed most of the tumors as adenomas. The study pathologist also observed a significantly increased ($p<0.05$) incidence of hepatocellular carcinoma in one group of female mice exposed to 16,000 ppm tetrachlorvinphos (0/99 and 5/46 for controls and high-dose animals, respectively).

Other Relevant Data

Tetrachlorvinphos has been shown to induce structural chromosomal aberrations and sister-chromatid exchange in a primary culture of mouse spleen cells (Amer and Aly, 1992). It was shown to increase chromosomal aberration frequencies in Chinese hamster ovary cells without metabolic activation (Hazleton Laboratories**,** 1989; as cited in CDPR, 1996). Tetrachlorvinphos is not mutagenic in *Salmonella typhimurium* or *Escherichia coli*, either with or without metabolic activation (Shell, 1978 as cited in CDPR, 1996; Ruiz and Marzin, 1997; Bartsch *et al*., 1980 as cited in IARC, 1983; Shirasu *et al.*, 1982). It did not increase mitotic gene conversion in stationary-phase cultures of *Saccharomyces cerevisiae* (Brooks *et al.*, 1982). Tetrachlorvinphos was reported to induce unscheduled DNA

synthesis in human embryo fibroblasts (Benigni and Dogliotti, 1980 as cited in IARC, 1983). It forms DNA adducts in mouse liver following intraperitoneal injection (Zayed *et al.*, 1983; Zayed *et al.*, 1984). Amer and Fahmy (1983) reported that tetrachlorvinphos induced micronuclei in mouse bone marrow after intraperitoneal and oral treatments. In *Aspergillus nidulans*, tetrachlorvinphos increased the frequency of mitotic crossing-over and non-disjunction without metabolic activation (Vallini *et al.*, 1983). Human lymphocytes treated with tetrachlorvinphos showed a slight increase in the number of metaphases with chromosomal aberrations (Kurinnyi and Pilinskaya, 1977). A slight increase in chromosomal aberrations in bone marrow cells was observed following treatment of mice with tetrachlorvinphos (Kurinnyi, 1975). Chromosomal aberrations were observed in root-tip meristems of *Vicia faba* (broad bean) plants treated with saturated and half-saturated solutions of tetrachlorvinphos in a detergent/water vehicle, but not those treated with aqueous vehicle (Amer and Mikhael, 1983).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over tetrachlorvinphos since it induced tumors at several sites in both sexes of mice and in female rats. Malignant liver tumors in male mice were caused by tetrachlorvinphos exposure in two separate studies. In female mice, malignant liver tumors were caused in one study, and combined malignant and benign liver tumors in another study. Also associated with tetrachlorvinphos exposure were kidney tumors in male mice in one study, and increased incidences of C-cell adenomas of the thyroid and cortical adenomas of the adrenal gland in a study in female rats. The level of concern is reinforced by a number of genotoxicity tests showing tetrachlorvinphos can induce chromosomal damages *in vitro* and *in vivo*, and by the observation of DNA adduct formation in mouse liver. Since tetrachlorvinphos was considered and rejected for listing by the Science Advisory Panel on April 26, 1991, this chemical will not be forwarded to the Carcinogen Identification Committee for consideration unless substantial relevant new data unavailable to the State's qualified experts in April 1991 become available.

There is a **HIGH** level of **concern over the extent of exposure,** since tetrachlorvinphos is a pesticide registered for control of insects in and on animals. The specific uses include various products and appliances for topical application of tetrachlorvinphos for the control of ectoparasites, and also in materials (oral larvicides) designed to be fed to animals to control fecal flies. Animals treated include cattle, horses, poultry and pets. These uses clearly involve much smaller quantities, and less general environmental exposures, to tetrachlorvinphos than the previous (prior to 1987) registrations for use on fruits and vegetables. However, there is still potential for significant exposure to specific subgroups within the population of California, either in an occupational setting for agricultural workers and others handling the products, or for consumers (and their children) who might use these materials on domestic pets. Further concerns for formerly registered uses of pesticides are the continued storage or use of old stocks of material and the environmental persistence of residues, although this may not be a major problem with tetrachlorvinphos. Some human exposure might also result from use as an oral larvicide.

References

Amer SM, Aly FAE (1992). Cytogenetic effects of pesticides: IV. Cytogenetic effects of the insecticides Gardona and Dursban. *Mutat Res* 279:165-170.

Amer SM, Fahmy MA (1983). Cytogenetic effects of pesticides: II. Induction of micronuclei in mouse bone marrow by the insecticide gardona. *Mutat Res* 117:329-336.

Amer SM, Mikhael E (1983). Cytological effects of the insecticide tetrachlorvinphos. *Toxicol Lett* **18**(3):231-4.

Brooks TM, Dean BJ, Hutson DH, Potter D (1982). Microbial mutation studies with tetrachlorvinphos (gardona). *Mutat Res* 105:211-222.

California Department of Pesticide Regulation (CDPR, 1996). *Summary of toxicological data - tetrachlorvinphos*. April 4, 1986. Updated January 17, 1996.

California Department of Pesticide Regulation (CDPR, 1999). Chemical ingredient database. Available at URL: http://www.cdpr.ca.gov/dprdatabase.htm.

California Department of Pesticide Regulation (CDPR, 1999). Summary of Pesticide Use Report Data, 1997, Indexed by Chemical.

International Agency for Research on Cancer (IARC, 1983). *IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans, miscellaneous pesticides*. Vol. 30. IARC, Lyon, France.

Inveresk Research International Ltd. (1993). Tetrachlorvinphos. 104 week dietary combined chronic toxicity/carcinogenicity study in rats. IRI Project No. 438403, Tranent, Scotland.

Kurinnyi AI (1975). Comparative study of the cytogenetic effect of certain organophosphorus pesticides. *Sov Genet* **11**(12):64-9.

Kurinnyi AI, Pilinskaya MA (1977). [Cytogenetic activity of the pesticides phthalophos, chlorophos and gardona in human lymphocyte cultures]. *Genetika (Moscow)* **13**(2):337-9.

National Cancer Institute (NCI, 1978). *Bioassay of tetrachlorvinphos for possible carcinogenicity*. Technical Report Series No. 33., U.S. Department of Health, Education, and Welfare, National Institutes of Health, DHEW publication no. (NIH) 78-833.

Parker CM, van Gelder GA, Chai EY, Gellatly JBM, Serota DG, Voelker RW, Vesselinovitch SD (1985). Oncogenic evaluation of tetrachlorvinphos in the $B6C3F_1$ mouse. *Fund Appl Toxicol* 5:840-854.

Ruiz MJ, Marzin D (1997). Genotoxicity of six pesticides by *Salmonella* mutagenicity test and SOS chromotest. *Mutat Res* 390:245-255.

Shirasu Y, Moriya M, Tezuka H, Teramoto S, Ohta T, Inoue T (1982). Mutagenicity screening studies on pesticides. *Proc 3rd Intl Conf Environ Mutagens*, pp.332-4

U.S. Environmental Protection Agency (U.S. EPA, 1997). *Health Effects Assessment Summary Tables. FY 1997 update*. Office of Research and Development, Office of Emergency and Remedial Response, Washington, DC. EPA-540-R-97-036.

Vallini G, Pera A, de Bertoldi M (1983). Genotoxic effects of some agricultural pesticides *in vitro* tested with *Aspergillus nidulans*. *Environ Poll (Ser A)* **30**:39-58.

Zayed SMAD, Mostafa IY, Adam Y, Hegazi B (1983). *In vivo* methylation of guanine by the organophosphorus insecticide tetrachlorvinphos. *J Environ Sci Health* B18(6):767-779.Zayed SMAD, Mostafa IY, Hegazi B (1984). Alkylation of urinary guanine in mice by the organophosphorus insecticide tetrachlorvinphos. *Exotoxicol Environ Safety* **8**:205-7.

CARCINOGENICITY DATA SUMMARY: METHYLPHENIDATE AND ITS HYDROCHLORIDE (RITALIN®)

Methylphenidate hydrochloride (Ritalin®; methyl α-phenyl-α-(2-piperidyl)acetate; CAS No. 298-59-9; CAS No. for methylphenidate is 113-45-1) is a drug used to treat narcolepsy and attention deficit hyperactivity disorders (ADHD). A derivative of piperidine, methylphenidate was first marketed in the 1960s (Diller, 1996). Ninety percent of the current total is made by Ciba-Geigy and is used in the United States (Leutwyler, 1996). It is a mild central nervous system stimulant, although its mode of action is not completely understood.

Records of production quotas maintained by the Drug Enforcement Agency show a steady output of approximately 1700 kilograms of legal methylphenidate HCl through the 1980's followed by a sharp increase in production in 1991 (Diller, 1996). From 1990 to May 1995, the annual U.S. production of methylphenidate increased by 500% to 10,410 kilograms (Diller, 1996). Data collected in 1993 found that ~72% of the 1.8 million persons receiving medication for ADHD were taking methylphenidate HCl (Diller, 1996). Ritalin® production levels (most recent data from 1995) suggest that 2.6 million people were taking Ritalin®, the vast majority of whom were children ages 5-12 (Diller, 1996). In comparison, according to the U.S. Census Bureau in 1994 there were 260,341,000 people in the U.S., with 45,165,682 of them being children from the ages of 5 to 17 (Byerly and Deardorff, 1995). These data illustrate that almost 1% of the entire U.S. population is taking Ritalin®. When looking at children alone this percentage is even higher. Furthermore, methylphenidate HCl also has abuse potential, especially among teenagers (Diller, 1996).

The average daily dosage of methylphenidate HCl for medical treatment is 10 mg, 2 or 3 times daily, with dosages above 60 mg and treatment below the age of five not recommended (PDR, 1994). The usual child dosage is 5 mg, twice daily (0.3-1 mg/kg). However, dosages are individualized according to the needs and responses of the patient, as well as on the basis of factors such as age and body weight. Exposure to methylphenidate can therefore vary from person to person. The average duration of treatment is 2 years when prescribed to elementary school-age children, 4 years for middle school-age children, and 7 years for high school-age children (NTP, 1995).

The U.S. FDA has reported that the NTP study produced a 'weak signal' of cancer-causing potential (U.S. FDA, 1996). U.S. FDA has indicated that as a follow-up, further short-term *in vivo* studies will be conducted, including: carcinogenicity in the neonatal mouse model, carcinogenicity in the P53 mouse model, metabolism and exposure studies, and 'possibly a repeat of the mouse lymphoma study'. U.S. FDA-sponsored epidemiological follow-up will include a case-control study comparing "the odds of prior exposure to Ritalin® in patients with hepatoblastoma with that in control subjects" and an evaluation of the feasibility of performing a cohort study in Ritalin-exposed children examining multiple cancer endpoints.

Carcinogenicity Data available:

Epidemiological studies

In a screening study of participants in a health plan in Oakland, California, taking a wide range of prescription drugs between 1969 and 1973 and followed up through 1984 (11-15 years later), 529 study subjects had received methylphenidate HCl (Selby *et al.*, 1989). No increased risk of cancer at any site was associated with methylphenidate HCl exposure. Fifteen cases of cancer were observed (32.7 cases expected), resulting in a standardized mortality ratio of 0.46 (negative association with $p<0.002$). Results were not adjusted for confounding due to smoking or alcohol consumption because of limited data available for the cohort. Occurrence of cancer in the general population was drawn from the California Resource for Cancer Epidemiology, the San Francisco Bay Area tumor registry, and the hospital's discharge abstracts.

In the NCI Surveillance, Epidemiology, and End Results (SEER) Pediatric Monograph, it was reported that "[t]he incidence of hepatoblastoma increased markedly during the 1975-1995 period" (Bulterys *et al.*, 1999; see animal evidence on hepatoblastoma below). Concern regarding this trend stems from possible correlation with the increased use of methylphenidate among children during this time period; however, according to the Monograph the increase occurred primarily in children less than four years of age and since methylphenidate is generally administered to children greater than six years of age, questions arise as to a possible association.

Animal bioassays

1. Rat long-term feeding studies: NTP, 1995. F344 rats (70/sex/dose) were fed 0, 100, 500, or 1,000 ppm methylphenidate HCl for up to two years. No significant increases in chemical-related neoplasm incidences were observed in male or female rats. The NTP concluded that there was no evidence of carcinogenic activity for methylphenidate hydrochloride in male or female rats under these conditions.

2. Mouse long-term feeding studies: NTP, 1995. $B6C3F_1$ mice (70/sex/dose) were fed 0, 50, 250, or 500 ppm methylphenidate HCl for 2 years. An increased incidence of hepatoblastoma occurred in high-dose male mice (0/50, 1/50, 1/50, 5/50; p=0.028) and increased incidences of hepatocellular adenoma occurred in both high-dose males (18/50, 18/50, 16/50, 29/50; p=0.02) and females (6/49, 10/48, 10/49, 28/50; p<0.01). The incidences of hepatocellular carcinoma were similar among control and exposed mice. The increase in combined hepatocellular adenoma, carcinoma and hepatoblastoma was significant in high-dose males (p=0.037) (24/50, 23/50, 26/50, 34/50). Combined hepatocellular adenoma and carcinoma was also significantly increased in high-dose females (p<0.001) (9/49, 11/48, 11/49, 30/50). The incidences of eosinophilic foci were increased in 500 ppm methylphenidate HCl-dosed male mice (6/50; 8/50; 9/50; 14/50) and female mice (3/49, 3/48, 8/49, 25/50). Based on the occurrence of hepatocellular neoplasms, the NTP concluded that there was some evidence of carcinogenic activity for methylphenidate HCl in male and female $B6C3F_1$ mice.

Other relevant data

Methylphenidate has been studied in two transgenic mouse models. Groups of 15 male and female $p53^{+/-}$ (6-8 weeks of age) and Tg.AC mice (7-9 weeks of age) were fed 0, 50, 250, or 500 ppm methylphenidate HCl for 24 weeks (Freeman *et al*., 1998; 1999). Microscopic examination revealed non-neoplastic liver lesions in both strains of mice, which were considered incidental and spontaneous, as no dose-related trend was evident. In Tg.AC mice, neoplastic and non-neoplastic odontogenic lesions were observed, but as these lesions are common background lesions in Tg.AC mice they were not considered related to methylphenidate exposure. No treatment-related neoplasms were observed in either strain of mice (Freeman *et al*., 1998; 1999). Spalding *et al*. (1999) have reviewed results from bioassays with Tg.AC mice for a number of liver specific nongenotoxic carcinogens. While some of these carcinogens are active in Tg.AC mice, others including methylphenidate are not.

Methylphenidate caused small, but significant, increases in the frequency of sister chromatid exchanges in lymphocytes from pediatric patients (Walker and Dumars, 1977). This result was only presented in abstract form and not followed-up in the peer-reviewed literature. Methylphenidate HCl was not mutagenic in *Salmonella typhimurium* with and without metabolic activation with S-9 (NTP, 1995) and it did not induce transformation of F344 rat embryo cells (Price *et al.*, 1978). It did, however, increase the frequency of sister chromatid exchanges and chromosomal aberrations in CHO cells (NTP, 1995). Methylphenidate was found to be non-mutagenic in L5178Y mouse lymphoma cells both with and without metabolic activation with S-9 (Rudd *et al.*, 1983) and did not induce unscheduled DNA synthesis when incubated with F344 male rat hepatocytes and tritiated thymidine (Mirsalis *et al.*, 1983).

Preliminary evaluation of carcinogenicity and exposure data

Although methylphenidate did not reach a level of concern sufficient to be placed on the candidate list, there is considerable carcinogenic concern from hepatocarcinogenic activity seen in male and female $B6C3F_1$ mice. A significant increase in the incidence of a rare malignant liver tumor type (hepatoblastoma) in male mice contributes to this level of concern. Concern is mitigated by the fact that the majority of the treatment-related hepatocellular tumors (as opposed to hepatoblastomas) were non-malignant tumors. Results from *in vitro* genotoxicity studies in rodent cells have been mixed with some positive findings of increased chromosome aberrations and SCE.

There is a **HIGH** level of **concern over the extent of exposure** since methylphenidate HCl is a commonly prescribed drug given on a long-term basis to children and others with attention deficit hyperactivity disorders and narcolepsy.

References

Bulterys M, Goodman MT, Smith M, Buckley JD (1999). Hepatic tumors. In: *Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975-1995*. Ries LAG, Smith MA, Gurney JG, Linet M, Tamra T, Young JL, Bunin GR (eds). National Cancer Institute, SEER Program. NIH Pub. No. 99-4649. Bethesda, MD. p. 93.

Byerly ER, Deardorff K (1995). National and State Population Estimates: 1990 to 1994, US Bureau of the Census, Current Population Reports, P25-1127, US Government Printing Office, Washington, DC, 1995.

Diller LH (1996). The run on Ritalin: Attention deficit disorder and stimulant treatment in the 1990s. *Hastings Center Report* **26**:12-18.

Dunnick JK, Elwell MR, Haseman JK (1996). Decreased incidence of spontaneous mammary gland neoplasms in female F344 rats treated with amphetamine, methylphenidate, or codeine. *Cancer Lett* **102**(1-2): 77-83.

Dunnick JK, Hailey JR (1995). Experimental studies on the long-term effects of methylphenidate hydrochloride. *Toxicology* **103**(2): 77-84.

Freeman GB, Reichelderfer D, Athey PM, Yarrington JT, Hejtmancik M, Eastin W, Vallant M, Chhabra R, Tennant RW (1999). Studies to determine the toxic potential of methylphenidate hydrochloride in transgenic mice. *Intl Journal of Toxicology* **18**(1):84.

Freeman GB, Reichelderfer D, Athey PM, Yarrington JT, Hejtmancik M, Eastin W, Vallant M, Chhabra R, Tennant RW (1998). Toxicity Evaluation of methylphenidate hydrochloride in transgenic mice. *Toxicological Sciences* **42**(1-S):72.

Leutwyler K (1996). Paying attention: The controversy over ADHD and the drug Ritalin is obscuring a real look at the disorder and its underpinnings. *Sci Amer* **275**:12;14.

Mirsalis J, Tyson K, Beck J, Loh F, Streinmetz K, Contreras C, Austere L, Martin S, Spalding J (1983). Induction of unscheduled DNA synthesis (UDS) in hepatocytes following in vitro and in vivo treatment [Abstract]. *Environ Mutagen* **5**:482.

National Toxicology Program (NTP, 1995). Toxicology and carcinogenesis studies of methylphenidate hydrochloride (CAS No. 298-59-9) in F344 rats and $B6C3F_1$ mice (feed studies). NTP Technical Report Series No. 439, NIH Publication No. 95-3355.

Physician's Desk Reference (PDR, 1994). Methylphenidate Hydrochloride. p. 897

Price PJ, Gregory EA, Skeen PC (1978). Ritalin, benzedrine and dexedrine do not transform F 1706 rat cells. *Cancer Lett* **5**:345-349.

Rudd CJ, Mitchell AD, Spalding J (1983). L5178Y mouse lymphoma cell mutagenesis assay of coded chemicals incorporation analyses of the colony size distribution [Abstract]. *Environ Mutagen* **5**:419.

Selby JV, Friedman GD, Fireman BH (1989). Screening prescription drugs for possible carcinogenicity: Eleven to fifteen years of follow-up. *Cancer Res* **49**:5736-5747.

Spalding JW, French JE, Eastin W, Mahler J, Bucher J, Furedi-Machacek M, Connor F, Tice RR, Tennant RW (1999). Recent retrospective and prospective studies in the Tg.AC and $p53^{+/-}$ transgenic mouse lines on chemicals completed in the two-year NTP bioassay. *Toxicological Sciences* **48**(1-S):253.

U.S. Food and Drug Administration (U.S. FDA, 1996). Ritalin: FDA Talk Paper T96-4, Jan 12, 1996.

Walker AP, Dumars KW (1977). Commonly used pediatric drugs, sister chromatid exchanges and the cell cycle [Abstract]. *Amer J Hum Genet* **29**:110A.