

**CHEMICALS MEETING THE CRITERIA FOR LISTING AS CAUSING
CANCER VIA THE AUTHORITATIVE BODIES MECHANISM**

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Reproductive and Cancer Hazard Assessment Section
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The chemicals listed in the table below meet the criteria for listing under Proposition 65 via the authoritative bodies listing mechanism as known to the State to cause cancer. The regulatory guidance for listing by this mechanism is set forth in Title 22, California Code of Regulations (CCR), Section 12306. For example, the regulations include provisions covering the criteria for evaluating the documentation and scientific findings by the authoritative body to determine whether listing under Proposition 65 is required.

The International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) are two of five institutions which have been identified as authoritative bodies for identification of chemicals as causing cancer for the purposes of Proposition 65 (22 CCR 12306(1)). One of these bodies has identified each of the chemicals in the table below as causing cancer. The Office of Environmental Health Hazard Assessment (OEHHA) has found that these chemicals have been “formally identified” as causing cancer according to the regulations covering this issue (22 CCR 12306(d)). The chemicals below are the subjects of reports published by the authoritative bodies that conclude that the chemicals cause cancer. Also, the documents specifically and accurately identify the chemicals and the documents meet one or more of the criteria outlined in 22 CCR 12306(d)(2).

OEHHA also finds that the criteria given in regulation for “as causing cancer” (22 CCR 12306(e)) have been satisfied for the chemicals in the table below. In making this evaluation, OEHHA relied upon the discussion of data by the authoritative bodies in making their findings that the specified chemicals cause cancer. A brief discussion of the relevant carcinogenesis studies providing evidence for the findings is presented below. The statements in bold reflect data and conclusions that satisfy the criteria for the sufficiency of evidence for carcinogenicity (22 CCR 12306(e)). The full citations for the authoritative body documents are given in this report.

Chemicals Meeting the Criteria for Listing As Causing Cancer

Chemical	CAS No.	Chemical Use	Reference
3,3'-Dimethoxybenzidine-based dyes metabolized to 3,3'-dimethoxybenzidine	-----	Used to color textiles, plastics, paper, rubber and leather goods.	NTP (2002)
3,3'-Dimethylbenzidine-based dyes metabolized to 3,3'-dimethylbenzidine	-----	Primary use in printing textiles.	NTP (2002)
Ethylbenzene	100-41-4	Industrial chemical used as a chemical intermediate; present in mixed xylenes and in gasoline.	IARC (1999)
Propylene glycol mono- <i>t</i> -butyl ether	57018-52-7	Used as a solvent for all-purpose cleaners, electronic chemicals, inks, adhesives, nail polish lacquers, and other water-reducible coatings.	NTP (2003a; 2003b)
Thiouracil	141-90-2	Used as a chemical intermediate and in metal plating. Previously used as an anti-thyroid drug.	IARC (2001)

3,3'-Dimethoxybenzidine-based dyes metabolized to 3,3'-dimethoxybenzidine

Increased incidence of malignant and combined malignant and benign tumors at multiple organ sites in male and female rats.

NTP (2002) concluded that dyes metabolized to 3,3'-dimethoxybenzidine are reasonably anticipated to be human carcinogens. This conclusion is based on the fact that 3,3'-dimethoxybenzidine is carcinogenic in male and female rats and that metabolism of 3,3'-dimethoxybenzidine-based dyes to release free 3,3'-dimethoxybenzidine is a generalized phenomenon that occurs in all animal species studied. 3,3'-Dimethoxybenzidine (*ortho*-dianisidine) was listed under Proposition 65 on January 1, 1988.

3,3'-Dimethoxybenzidine-based dyes are synthesized by linking various chromophores to the base chemical by azo linkages. Regardless of the chromophore used, the azo bonds are easily broken by chemicals or enzymes to release free 3,3'-dimethoxybenzidine and chromophore. Gastrointestinal tract bacteria are thought to be the primary agents of this cleavage (NTP, 2002). A number of bacteria catalyze this reaction, including *Escherichia coli*, found in the human gastrointestinal tract.

3,3'-Dimethoxybenzidine causes an increased incidence of tumors of the Zymbal gland, liver, intestine, skin and oral cavity in male and female rats. In addition, in male rats, 3,3'-dimethoxybenzidine caused prepuptial gland carcinoma, adenocarcinoma of the small intestine and mesothelioma. In female rats, 3,3'-dimethoxybenzidine caused an increased incidence of clitoral gland adenoma and carcinoma, mammary adenocarcinoma and uterus or cervix adenoma and carcinoma.

In its evaluation, NTP (2002) cited C.I. Direct Blue 15 as an example of a representative 3,3'-dimethoxybenzidine-based dye. C.I. Direct Blue 15 is also carcinogenic in male and female rats and causes the same spectrum of tumors: skin, Zymbal gland, liver, oral cavity, gastrointestinal tract in male and female rats; tumors of the preputial gland in male rats and clitoral gland in female rats.

3,3'-Dimethylbenzidine-based dyes metabolized to 3,3'-dimethylbenzidine

Increased incidence of malignant and combined malignant and benign tumors at multiple organ sites in male and female rats.

NTP (2002) has concluded that dyes metabolized to 3,3'-dimethylbenzidine are reasonably anticipated to be human carcinogens. This conclusion is based on the fact that 3,3'-dimethylbenzidine is carcinogenic in male and female rats and that metabolism of 3,3'-dimethylbenzidine-based dyes to release free 3,3'-dimethylbenzidine is a generalized phenomenon that occurs in all animal species studied. 3,3'-Dimethylbenzidine was listed under Proposition 65 on January 1, 1988.

3,3'-Dimethylbenzidine-based dyes are synthesized by linking various chromophores to the base chemical by azo linkages. Regardless of the chromophore used, the azo bonds are easily broken by chemicals or enzymes to release free 3,3'-dimethylbenzidine and chromophore. Gastrointestinal tract bacteria are thought to be the primary agents of this metabolism (NTP, 2002). A number of bacteria catalyze this reaction, including *Escherichia coli*, found in the human gastrointestinal tract.

3,3'-Dimethylbenzidine causes an increased incidence of tumors of the Zymbal gland, liver, intestine, skin and oral cavity in male and female rats. In addition in male rats, 3,3'-dimethoxybenzidine causes preputial gland carcinoma, adenocarcinoma of the small intestine and mesothelioma. In female rats, 3,3'-dimethylbenzidine causes an increased incidence of clitoral gland adenoma and carcinoma, mammary adenocarcinoma and uterus or cervix adenoma and carcinoma.

NTP (2002) cited C. I. Acid Red 114 as an example of a representative 3,3'-dimethylbenzidine-based dye. Like 3,3'-dimethylbenzidine, C. I. Acid Red 114 induces tumors of the skin, Zymbal gland, liver, oral cavity, and gastrointestinal tract in male and female rats and tumors of the preputial gland in male rats and clitoral gland in female rats, as well as tumors at other tissue sites.

Ethylbenzene (CAS No. 100-41-4)

Increased incidence of combined malignant and benign tumors in male rats to an unusual degree with respect to tumor site.

IARC (1999) has concluded that there is sufficient evidence for the carcinogenicity of ethylbenzene in experimental animals and has classified ethylbenzene in Group 2B, possibly carcinogenic to humans. IARC concluded that in mice, ethylbenzene increased

the incidence of lung adenomas in males and liver adenomas in females; and, in male rats, ethylbenzene increased the incidence of renal tubule adenomas and carcinomas. IARC also concluded that a metabolite of ethylbenzene increased renal tubule adenomas in male rats. The relevant studies evaluated by IARC are summarized below.

NTP (1999) exposed Fischer 344/N rats (50 rats/group/sex) to ethylbenzene by inhalation for six hours per day, five days per week for 104 weeks. In male rats, the incidence of renal tubule carcinoma was 0/50, 0/50, 1/50 and 3/50 [$p < 0.025$, trend test] for control, low-, mid- and high-dose groups, respectively and exceeded the incidence of renal tubule carcinoma in the NTP historical database for two-year inhalation studies with chamber controls (0/652). The incidence of combined renal tubule adenomas or carcinomas in high-dose males (single section: 0/50, 3/50, 3/50, and 7/50 (14%) [$p < 0.01$]) was statistically significant and also exceeded the incidence of renal tubule adenoma or carcinoma in the NTP historical database for two-year inhalation studies with chamber controls (6/652 (1%)). After step-sectioning, the incidence of renal tubule adenoma or carcinoma in combined single and step-sections in male rats was 3/50, 5/50, 8/50, 21/50 (42%) [$p < 0.01$]. Thus, the incidence of combined renal tubule adenoma and carcinoma in male F344/N rats treated with ethylbenzene was increased to an unusual degree with respect to tumor site. After step-sectioning, the incidence of renal tubule adenomas was also significantly greater in high dose female rats compared to control females (combined incidence of single and step sections: 0/50, 0/50, 1/50 and 8/49 [$p < 0.01$]).

NTP (1999) exposed B6C3F₁ mice (50 rats/group/sex) to ethylbenzene by inhalation for six hours per day, five days per week for 103 weeks. In female mice, there was a statistically significant increase in the incidence of combined hepatocellular adenoma or carcinoma in high dose animals (13/50, 12/50, 15/50, 25/50 [$p < 0.05$]). In male mice, the incidence of alveolar/bronchiolar adenomas was significantly increased in high-dose animals compared to controls (5/50, 9/50, 10/50, 16/50 [$p < 0.01$]), as was the incidence of combined alveolar/bronchiolar adenoma or carcinoma (7/50, 10/50, 15/50, 19/50 [$p < 0.01$]). IARC (1999) noted that although significant increases in tumor incidence were observed in both male and female mice, the increases were within historical control ranges in both cases.

IARC (1999) also reviewed earlier NTP bioassays with the ethylbenzene metabolite, 1-phenylethanol (α -methylbenzyl alcohol). In those studies, NTP (1990) exposed male and female B6C3F₁ mice and Fischer 344 rats (50 animals/group/sex) to 1-phenylethanol via corn oil gavage for five days per week for 103 weeks. No increases in tumor incidence were seen in mice. In both male and female rats, poor survival was noted. IARC (1999) noted that the poor survival in males (and females) was due to accidental deaths related to gavage. Referring to the poor survival in the rats, NTP (1990) states that "excessive mortality in the two-year study in male rats reduced the sensitivity of this study for the detection of a carcinogenic response." An increase in renal tubule adenoma was observed in male rats (0/50, 1/50, 5/50 for control, low- and high-dose animals, respectively), and a tubular cell adenocarcinoma was found in one low-dose male rat. After step-sectioning, the combined incidence of renal tubule adenoma or adenocarcinoma was 1/49, 13/41 [$p < 0.001$] and 14/28 [$p < 0.001$].

Propylene glycol mono-*t*-butyl ether (CAS No. 57018-52-7)

Increased incidence of a rare malignant tumor in male mice and increased incidence of combined malignant and benign tumors in male and female mice.

The NTP (2003a; 2003b) has concluded that there is clear evidence of the carcinogenic activity of propylene glycol mono-*t*-butyl ether in male and female B6C3F₁ mice.

NTP (2003a) exposed B6C3F₁ mice and F344/N rats to propylene glycol mono-*t*-butyl ether via inhalation for two years. In male mice exposed to propylene glycol mono-*t*-butyl ether, there was a statistically significant [$p=0.028$] incidence of hepatoblastoma (0/5, 0/49, 1/50, 5/50 for control, low-, mid-, and high-dose groups, respectively). NTP (2003a) notes that hepatoblastomas are “very rare tumors with an extremely low spontaneous incidence in mice.” No hepatoblastomas have been observed in 250 historical chamber control male mice in the current NTP historical database. In addition, the incidences of hepatocellular adenoma (18/50, 23/49, 26/50 and 36/50) and combined hepatocellular adenoma or carcinoma (25/50, 26/49, 33/50, 41/50) increased significantly in a dose-related fashion and were significantly [$p<0.001$] greater in high-dose males than in chamber controls. In female mice, the incidences of hepatocellular adenoma (14/49, 8/50, 10/50 and 37/49) and combined adenoma or carcinoma (18/49, 14/50, 16/50, 41/49) were significantly [$p<0.001$] greater in the high-dose group compared to the control group. The incidence of hepatocellular carcinoma was also increased (4/49, 8/50, 7/50, and 10/49) although the incidence in the high-dose group was not significantly greater than that in the control group [$p=0.071$]; however, the incidence in the high dose group (20%) exceeded the NTP historical control range (8%-12%). Two hepatoblastomas were observed in high-dose female mice. The NTP chamber historical control incidence for these rare tumors is 0/248 in female mice.

In exposed male rats, NTP (2003a) found that there was equivocal evidence of carcinogenicity based on marginally increased incidence of renal tubule and liver neoplasms.

Propylene glycol mono-*t*-butyl ether was mutagenic in *Salmonella typhimurium* strain TA97 in the absence of liver S9 activation enzymes, but was not mutagenic in strains TA98, TA100 and TA1535 with or without S9 liver enzymes. NTP (2003a) found that the mutagenic activity observed exclusively in strain TA97 without S9 enzymes “indicates that propylene glycol mono-*t*-butyl ether operates through a frameshift mechanism in this test system ...” NTP (2003a) noted that ethylene glycol monobutyl ether was also found to be mutagenic exclusively in the closely-related *S. typhimurium* strain TA97a by one laboratory, although the response was not duplicated in another laboratory.

NTP’s findings were published in a draft technical report and were subsequently reviewed by an external advisory committee in a public meeting and accepted at the May 22, 2003 NTP Board of Scientific Counselors’ Technical Reports Review Subcommittee Meeting, as reported on the NTP website at <http://ntp-server.niehs.nih.gov> (NTP, 2003c).

Thiouracil (CAS No. 141-90-2)

Increased incidence to an unusual degree of malignant thyroid tumors in male and female rats. In addition, hepatomas and metastatic thyroid tumors were increased in mice.

IARC (2001) has concluded that there is sufficient evidence for the carcinogenicity of thiouracil in experimental animals and has classified thiouracil in Group 2B, possibly carcinogenic to humans. The relevant studies evaluated by IARC are summarized below.

Fears *et al.* (1989) fed male and female F344 rats a diet containing thiouracil (23-24 animals/group/sex) for 104 weeks. A control group consisted of 214 male and 214 female rats. The incidence of malignant thyroid follicular-cell tumors was 5/214, 6/24 [$p=0.0002$], 14/24 [$p=10^{-12}$] and 5/24 [$p=0.0002$] in males and 5/214, 2/23, 6/24 [$p=0.0002$] and 18/24 [$p\leq 10^{-17}$] in females for control, low-, mid- and high-dose groups, respectively.

Gorbman (1947) fed three strains of mice (A, C57 and I)[sex not specified] a diet containing 0 or 0.1% thiouracil for various periods up to 81 weeks. Group sizes of treated and control mice, respectively, were as follows for each strain: Strain A: 28 and 39; C57: 29 and 51; Strain I: 24 and 35. After 40 days of treatment, thyroid follicular-cell hyperplasia was present in 69 treated mice (three strains combined). The hyperplasia developed into follicular cystic or nodular lesions after 180 days. Pulmonary foci very similar to the hyperplastic thyroid tissue were seen in seven treated Strain A mice. While the author of this paper interpreted the lesions to be non-malignant, IARC (2001) noted that under current histopathological criteria, the thyroid and pulmonary lesions described in this study might be diagnosed as thyroid neoplasia and metastases of thyroid neoplasia.

Dalton *et al.* (1948) fed 143 female C3H mice a basal diet or a diet containing thiouracil. IARC (2001) reported that the number of mice in the treated and control groups was approximately equal. Animals were killed at selected intervals or when moribund. Thyroid follicular-cell hyperplasia developed in treated mice during the first 12 months of the study although no neoplasia was diagnosed. However, of those animals treated for 362-464 days, pulmonary metastases of thyroid tissue were observed in 10/23 mice. Although the authors concluded that this was 'benign metastasizing thyroid tissue,' IARC (2001) noted that under current histopathological criteria, the pulmonary lesions might be regarded as metastases of thyroid neoplasia.

Casas (1963) fed thiouracil to two strains of male and female mice (C3H and an inbred strain designated TM) for 17 months. IARC (2001) noted that the initial numbers of animals in this study were not specified. In C3H mice, hepatomas were observed in both male (12/13) and female (14/16) mice treated with thiouracil. The incidence of hepatomas in the control groups were 2/32 and 0/24, for males and females, respectively. No hepatomas were observed in treated or control TM mice.

REFERENCES

- Casas CB (1963). Induction of hepatomas by thiouracil in inbred strains of mice. *Proc Soc Exp Biol Med* **113**:493-494.
- Dalton AJ, Morris Hp, Dubnick CS (1948). Morphologic changes in the organs of female C3H mice after long-term ingestion of thiourea and thiouracil. *J Natl Cancer Inst* **9**:201-223.
- Fears TR, Elashoff RM, Schneiderman MA (1989). The statistical analysis of a carcinogen mixture experiment. III. Carcinogens with different target systems, aflatoxin B₁, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, lead acetate, and thiouracil. *Toxicol Ind Health* **5**:1-22.
- Gorbman A (1947). Thyroidal and vascular changes in mice following chronic treatment with goitrogens and carcinogens. *Cancer Res* **7**:746-758.
- International Agency for Research on Cancer (IARC, 1999). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. Volume 77: 227-266. *Some Industrial Chemicals*. IARC, Lyon France.
- International Agency for Research on Cancer (IARC, 2001). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. Volume 79. *Some Thyrotropic Agents*. IARC, Lyon France.
- National Toxicology Program (NTP, 1990). *Toxicology and Carcinogenesis Studies of α -methylbenzyl alcohol, CAS No. 98-85-1 in F344/N Rats and B6C3F₁ Mice (Gavage Studies)*. NTP Technical Report Series No. 369. NIH Publication No. 89-2824. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NTP, Research Triangle Park, NC.
- National Toxicology Program (NTP, 1999). *Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F₁ Mice (Inhalation Studies)*. NTP Technical Report Series No. 466. NIH Publication No. 99-3956. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NTP, Research Triangle Park, NC.
- National Toxicology Program (NTP, 2002). *Tenth Report on Carcinogens*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NTP, Research Triangle Park, NC.
- National Toxicology Program (NTP, 2003a). *Toxicology and Carcinogenesis Studies of Propylene Glycol Mono-*t*-butyl ether (CAS No. 57018-52-7) in F344/N Rats and B6C3F₁ Mice and a Toxicology Study of Propylene glycol Mono-*t*-butyl ether in male NBR rats*. Board Draft. NTP Technical Report Series No. 515. NIH Publication No. 03-4449. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NTP, Research Triangle Park, NC.

National Toxicology Program (NTP, 2003b). *Actions on Draft Technical Reports by the NTP Board of Scientific Counselors Technical Reports Review Subcommittee on May 22, 2003*, as reported on the NTP website at <http://ntp-server.niehs.nih.gov>.