Candidates for Proposition 65 Listing via the Authoritative Bodies Mechanism Found Not to Meet the Scientific Criteria (22 CCR 12306(g))

Office of Environmental Health Hazard Assessment
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The U.S. Environmental Protection Agency (U.S. EPA), an authoritative body for purposes of Proposition 65 (22 CCR Section 12306(l)), identifies chemicals as causing developmental or reproductive toxicity in implementing its Toxic Release Inventory (TRI) program (*i.e.*, Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA)). On this basis the U.S. EPA, in 1994, added a number of chemicals to the TRI list and published its findings in the *Federal Register* (**59:**1788-1859, 1994 and **59:**61432-61485, 1994). The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed the bases for these TRI chemical additions in the context of the regulatory criteria governing Proposition 65 listing via the authoritative bodies mechanism (Title 22, California Code of Regulations, Section 12306 (22 CCR 12306)).

OEHHA determined for several TRI chemicals that the 22 CCR 12306 regulatory criteria were met and is in the process of placing these chemicals on the Proposition 65 list of chemicals known to cause reproductive toxicity. OEHHA has determined that these criteria have not been met for some of the chemicals added by U.S. EPA in 1994 to the TRI list on the basis of reproductive or developmental toxicity. These chemicals are listed in Table 1. In each case the scientific criteria for "as causing reproductive toxicity" given in regulation (22 CCR 12306(g)) were not satisfied, as described below.

In accordance with 22 CCR 12306(i), three of the chemicals in Table 1, quizalofop-ethyl, sodium nitrite and triphenyltin hydroxide, will be referred to the Developmental and Reproductive Toxicant Identification Committee of the OEHHA Science Advisory Board because this determination was made subsequent to the issuance of a notice of intent to list (*California Regulatory Notice Register (CRNR)*, February 26, 1999; December 4, 1998; and January 21, 1999, respectively). Therefore, at a future meeting, the Committee will opine whether "the chemical has been clearly shown through scientifically valid testing according to generally accepted principles" to cause reproductive toxicity. A *CRNR* notice for one other chemical in Table 1 (naled (November 20, 1998)) announced that the regulatory criteria for listing may have been met. However, because a notice of intent to list was not issued, this chemical will not be referred to the Committee for its review.

Table 1: TRI chemicals not meeting the scientific criteria (22 CCR 12306(g)) for authoritative bodies listing as causing reproductive toxicity under Proposition 65

Anilazine (000101-05-3)
Naled (000300-76-5)
Quizalofop-ethyl (076578-14-8)
Sodium Nitrite (007632-00-0)
Triphenyltin hydroxide (000076-87-9)

Anilazine (CAS No. 000101-05-3)

U.S. EPA (*Federal Register* 59(8):1798, 1994) bases its finding of developmental toxicity on one rat and one rabbit developmental study. The citation for both is the 1993 'Tox-Oneliner' database, which is no longer available. The 1997 Tox-Oneliner for anilazine describes the results of three developmental toxicity studies, two in rats and one in rabbits. One of the rat studies appears to correspond to the rat study described in the 1993 Tox-Oneliner, in which no effects were identified even at the highest dose tested. Both the second rat study and the rabbit study have notations stating, "Study is missing so much information, no conclusions will be drawn."

Naled (CAS No. 000300-76-5)

The U.S. EPA based its finding of female reproductive toxicity on a two-generation reproduction study of naled in rats in which decreased litter size, survival, and pup body weight were observed in the higher dose group. The only effect associated with pre-natal exposures was a statistically significant decreased litter size at birth in the F2b litter. However, 13% of the male and female F1 parents of this litter, died of toxicity-related causes. This level of parental toxicity is considered "excessive" under the U.S. EPA Guidelines for Developmental Toxicity Risk Assessment (*Federal Register* 56(234): 63798-63826, 1991). Due to the excessive maternal toxicity, this study is of limited usefulness for judging the impact of naled on litter size. With respect to postnatal findings, statistically significant decreased postnatal pup survival was observed in the F1 and F2b litters, and statistically significant decreased postnatal weight was observed in the F1 litter. However, all of these followed postnatal exposure of the lactating dams to the chemical. Under the U.S. EPA Guidelines for Developmental Toxicity Risk Assessment, the definition of developmental toxicity includes effects resulting from exposure during the postnatal developmental period, while under the current interpretation, the Proposition 65 statute precludes listing on the basis of developmental effects resulting from postnatal exposures.

Quizalofop-ethyl (CAS No. 076578-14-8)

Addition of quizalofop-ethyl to the TRI list on the basis of reproductive toxicity relied upon a single six month feeding study in beagle dogs (Subchronic Toxicity Study in Dogs with NC-302 by Oral Administration for 26 Weeks. Nippon Experimental Medical Research Institute, 1982). Histopathological examination of testicular tissue at the end of the study revealed atrophy of seminiferous tubules in two of six male dogs in the highest dose group tested. This change was described as not so severe as to cause disturbance in spermatogenesis, and was not statistically significant by pair-wise comparison with the control group. No adverse effects in testes of control, lowdose or middose males were reported. A second study in beagle dogs exposed to the same dose by the same route, but for a duration of one year, failed to find any adverse testicular effects upon histopathological examination (NC-302: 52 Week Oral (Dietary Administration) Toxicity Study in the Beagle Dog. Nissan Chemical Industries, 1985). The small number of animals exhibiting the effect and the minimal severity of the effect in the first study, and the failure to replicate the effect

with a longer exposure to the same dose level suggests that the original finding may not be treatment related.

Sodium Nitrite (CAS No. 007632-00-0)

U.S. EPA (*Federal Register* 59:1788-1859, 1994) added sodium nitrite to the TRI list of reported chemicals on the basis of the following information:

> "230. Sodium nitrite (CAS No. 007632-00-0) (CERCLA) (Ref. 8). Sodium nitrite causes conversion (oxidation) of hemoglobin to methemoglobin. Methemoglobin cannot combine reversibly with oxygen and its formation can cause anemic hypoxia which may lead to intense cyanosis. Infants are particularly susceptible to this effect because of their higher stomach pH, immature enzyme systems, the reduced capacity of newborn erythrocytes to reduce methemoglobin to hemoglobin, and the increased rate of nitrite-induced oxidation of fetal hemoglobin to methemoglobin (approximately twice the rate of adult hemoglobin oxidation). Coma and methemoglobinemia/ carboxyhemoglobinemia were reported in a human that received sodium nitrite (71 mg/kg) orally. In animal studies, methemoglobinemia was reported in dogs that received an intravenous dose of 30 mg/kg sodium nitrite and in rats administered a 10 mg/kg dose of sodium nitrite subcutaneously."
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> "Fetotoxicity (fetal death) was reported following oral exposure of pregnant rats to sodium nitrite (30 mg/kg/day) during gestation days 1 through 22. In mice exposed orally to 80 mg/kg/day during gestation days 6 to 15 there was increased preimplantation loss and fetal death, and in mice exposed to a lower dose (20 mg/kg/day) during gestation days 1 to 14, abnormalities of the blood or lymphatic system were reported in offspring. In offspring of rats orally exposed to 26 to 256 mg/kg/day during pregnancy (gestation days 1 through 22) and/or during lactation (20 to 21 days after birth), effects on growth including biochemical and/or metabolic changes were noted."

Neonatal methemoglobinemia is a serious, life-threatening, and well-documented toxic consequence of nitrite exposure. It is also a toxic endpoint which is encompassed by U.S. EPA's definition of developmental toxicity as:

> "…adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation."

However, as currently interpreted the Proposition 65 statute precludes listing on the basis of developmental effects resulting solely from postnatal exposures. Therefore, in determining whether the criteria specified in 22 CCR 12306(g) have been met, only data that pertain to prenatal exposures are considered. Thus, methemoglobinemia in exposed human neonates resulting from postnatal exposures do not provide a basis for listing a chemical as causing reproductive toxicity under Proposition 65.

The four animal studies cited by U.S. EPA in which exposures occurred that were entirely prenatal all provide some indication of a potential hazard posed by sodium nitrite to the developing organism. These four studies were conducted by various protocols, focused on diverse endpoints, and do not support each other in identifying a particular target of sodium nitrite toxicity. Three of the studies cited have limitations in design and reporting such that they contribute little to hazard identification. In a developmental toxicity study in rats (Schuval and Gruener, *Am J Pub Hlth*, 62(8):1045-1052, 1972.), the only developmental effect mentioned in the study report was, "[t]he litters in the control group contained 10 fetuses, 9.5 in Group I and 8.5 fetuses in Group II." Statistical analyses were not provided and the statistical significance of the result was not reported. In a developmental toxicity study in mice (Globus and Samuel, *Teratology* 118:367-378, 1978), using a single experimental dose (0.5 mg/kg/day), erythropoiesis was evaluated as numbers of either hepatic erythrocytes or all fetal hepatic red blood cells as a percent of total hepatic blood cells. The percentages of these cells were significantly increased in day 14 and 16 embryos, but not day 18 embryos. These effects could not be correlated with any actual increase in peripheral RBC counts, hence the functional significance of the observed changes is unclear. In another developmental toxicity study in rats (Roth *et al.*, *Fund Appl Toxicol* 9:668-677, 1987), the text makes statements pertaining to the cross-fostering portion of the study which appear to indicate some effect of prenatal exposure on early pup growth. The statements are not supported by tabular or graphical presentation of actual data.

In evaluating the remaining study (Shiobara, *Nichi Eishi* 42(2):836-846, 1987.), published in Japanese, OEHHA initially relied on the English summary and tables, which reported reduced litter size. Subsequently, detailed review of a full translation of the text of the study paper, casts significant doubt over the conclusions that can be drawn from the study. The reported findings of decreased litter size among treated animals may not be the result of treatment. Since treatment began on day six of gestation, the same day implantation occurs, and since there was a significant increase in pre-implantation loss, the change in litter size may be an artifact of this non-treatment-related variation in implantation frequency. Thus, the body of evidence related to prenatal exposures cited by U.S. EPA is not of sufficient quantity to satisfy the regulatory criteria for listing under the authoritative bodies provision (i.e., 22 CCR 12306(g)).

<u>Triphenyltin hydroxide (CAS No. 000076-87-9)</u>

A single study of developmental toxicity in rats formed the basis for TRI identification of triphenyltin hydroxide (Winek *et al.*, *Toxicol. Annu.* 3:281-96, 1979). Three experimental groups of six timed mated females per group received the chemical orally on days 1-7, 8-14 and 14-18 of gestation, respectively. There were two control animals per treatment group. Maternal mortality in the second and third groups (17% and 33%, respectively) exceeded the U.S. EPA definition of minimal maternal toxicity. The first treatment group, which had no maternal mortality, showed a complete failure of pregnancy, with no fetuses or resorption sites, and no endometrial proliferation or increased vascularity. These effects are indicative of female reproductive toxicity rather than developmental toxicity, the endpoint identified by U.S. EPA.