Description of a Proposed Reference Dose
Resorcinol

1. INTRODUCTION

This paper supports a submission of a formal rule-making petition to the Pennsylvania Environmental Quality Board (EQB) by describing a toxicity factor that can be derived from available data and be applied to the MSC algorithms provided in Chapter 250 of Act 2 (Title 25 Pennsylvania Code Subchapter C). The following sections of this paper apply the U.S. Environmental Protection Agency's methodology (EPA, 1993; Dourson, 1994) to develop a toxicity factor referred to as a "Reference Dose" (RfD). An RfD is defined as:

"An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." (Integrated Risk Information System [IRIS], 2001)

2. PHYSICAL AND CHEMICAL PROPERTIES OF RESORCINOL

Resorcinol is the common name for m-dihydroxybenzene (CAS # 108-46-3; also known as 1,3-benzenediol or m-hydroxyphenol); a synthetic phenolic chemical, shown in Figure 1. It is a crystalline solid produced in large quantities by sulfonating benzene with fuming sulfuric acid and fusing the resulting benzene-disulfonic acid with caustic soda.

Resorcinol is not likely to exist in air at high concentrations due to its low vapor pressure (vapor pressure 0.000489 mm Hg at 25° C; molecular weight 110.11; Henry's law constant 8.1×10^{-11} atm-m^3/mole). The compound has a faint characteristic aroma.

Resorcinol is very soluble in water (71,700 milligrams per liter at 25° C). Both the solid crystals and aqueous solutions of resorcinol become pink on exposure to light or upon contact with iron.

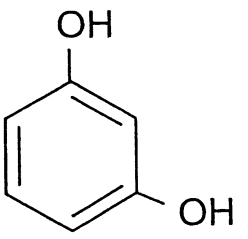

Figure 1. Structure of Resorcinol

3. USES OF RESORCINOL

The use of resorcinol as a therapeutic agent was first reported more than 100 years ago (Andeer, 1884). Today, resorcinol is used in pharmaceutical preparations for the topical treatment of skin conditions such as acne, seborrheic dermatitis, eczema, psoriasis, corns, calluses, and warts, and has recently been approved for use in chemical "peels", a dermatological treatment for acne scarring, irregular pigmentation, and actinic keratoses. In general, these products represent combinations of resorcinol with either salicylic acid or sulfur. Some of the common commercial products containing resorcinol include:

Acne/Skin products:	Clearasil®, Differin®, OxyClean®Noxema® , Ionil®, Acnomel® Cream, Bensulfoid®Cream, Sulforcin®, Rezamid®
Wart removal products:	Freezone®, Compound W®
Surgical sutures:	Duofilm®

Resorcinol is also used in the rubber industry where it is combined with formaldehyde to produce resins used to make rayon and nylon amenable to impregnation with rubber for tire bodies and reinforced tread rubber for use as conveyer and drive belts. It is also used to produce adhesives used in high performance lumber composites (laminated structural beams). Resorcinol is also used as a chemical intermediary in the manufacture of ultraviolet light screening agents, explosives, dyestuffs, photographic developers and cosmetics.

4. PHARMACOKINETICS OF RESORCINOL

The results of pharmacokinetic studies in rats, rabbits and humans, described below, suggest that resorcinol administered by either the oral, dermal, or subcutaneous route will be absorbed, rapidly metabolized, and excreted primarily as glucuronide conjugates in the urine. Few differences are seen between mammalian species, including man, in the qualitative or quantitative handling of the compound. Thus, animal toxicity testing of resorcinol should provide good information on potential effects in humans.

4.1. ABSORPTION

Studies in several species, including humans, indicate that resorcinol is readily absorbed from the gastrointestinal tract and can be absorbed to a lesser extent through skin.

An early study of the disposition of orally administered resorcinol in rabbits was reported by Garton and Williams (1949). In this study, approximately 76% of the administered dose was recovered from urine within 24 hours of dosing. This suggests that at least this proportion of the resorcinol dose was systemically absorbed. Kim and Matthews (1987) investigated the fate of [^{14}C]-resorcinol after oral administration to male and female rats. Greater than 90% of the dose was recovered in urine within 24 hours, again suggesting high systemic absorption from the gastrointestinal tract.

The ability of resorcinol to penetrate human skin is apparent in several case reports of acute toxicity in humans treated with topical compounds (i.e., applied to the skin) containing high concentrations of resorcinol. In most of these cases, resorcinol had been applied as an ointment or solution to ulcerated or otherwise damaged skin (Klein, et al, 1956; Thomas and Gisburn, 1961; Lundell and Normand; 1973) and might therefore be expected to more efficiently absorbed than would be the case for intact skin.

Studies have investigated dermal absorption of resorcinol in humans to determine its safety as a topical pharmaceutical. Yeung et al. (1981) and Yeung, et al (1983) report on three male subjects who received twice daily exposures to 20 ml of 2% resorcinol applied to the face and upper body, six days a week for four weeks. Urine and plasma were analyzed for the presence of free resorcinol and metabolites. The detection limit of the method was 0.1 μg/ml. No resorcinol or metabolites were detected in plasma. Metabolites (conjugates) of resorcinol were detected in a 24-hour urine sample taken after two weeks of treatment and corresponded to between approximately 0.5 and 3 % of the applied dose. This suggests resorcinol was absorbed into the system through the skin, although total quantities cannot be determined from this experimental design.

In vitro skin penetration studies were also conducted by Yeung et al. (1983). The studies utilized excised human skin in a permeation apparatus. *In vitro* skin penetration occurred at a rate of 0.86 μg/cm^2/hour. The authors state that this rate was in good agreement with the *in vivo* penetration rate of 0.37 μg/cm^2/hour, calculated using the dose recovered from urinary excretion experiment described previously. These results indicate resorcinol may penetrate intact skin, although it is likely that this route of exposure is less efficient than the oral route.

4.2. DISTRIBUTION

No studies of resorcinol tissue distribution have been conducted in humans.

Oral exposure to carbon-14 labeled resorcinol ([^{14}C]-resorcinol) at a dose of 112 mg per kilogram body weight (mg/kg) in corn oil resulted in general distribution of radioactivity without appreciable

accumulation in any particular organ of rats (Kim and Mathews, 1987). The majority of the dose was detected in excreta (90% in urine, 2% in feces) within 24 hours of dosing. These findings lead the authors to conclude that the compound would be rapidly cleared and not accumulate in any organ.

Merker, et al. (1982) dosed rats with [^{14}C]-resorcinol by the subcutaneous injection route, either as a single dose of 10, 50, and 100 mg/kg, or a single 50 mg/kg dose following 14-day and 30-day "pretreatments" with unlabelled resorcinol (100 mg/kg per day, given as two subcutaneous doses of 50 mg/kg). Urine was collected until the time of sacrifice and plasma and organs were taken for analysis in animals killed one, three, and 24 hours after dosing (a six-hour sacrifice was also conducted in the experiment with pretreated animals). As part of the 14- and 30-day multiple dose studies, Merker, et al. (1982) collected blood samples from the retro-orbital sinus every 15 minutes for one hour and at two, three, and six hours post-dosing.

It was found that plasma resorcinol peaked at 15 minutes after subcutaneous administration and ninety percent was eliminated within two hours. Similar to other reports, these investigators found low-level distribution to many organs. However, they report a proportionally greater content of the compounds[1] in liver and kidney at the shorter time periods, particularly in pretreated animals. Low dose (10mg/kg) distribution studies showed 62% resorcinol equivalents recovered in the urine at 1 hour, 7.1% in the gastrointestinal tract, 2.3% in the muscle, 0.72% in the kidneys, and 0.48% in the liver. At the 50 and 100 mg/kg dose levels, only 0.2% to 0.3% of the administered radioactivity was present in any organ at one hour post dosing, with the liver and kidneys containing the highest percentages. After three hours, only trace amounts were detected in any of the tissues sampled.

Merker, et al. (1982) suggest a biphasic elimination from plasma, with a "fast" half-life ($t_{1/2}$) of 20 to 30 minutes and slower rate with $t_{1/2}$ of approximately 5 to 11 hours. No hypothesis for the biphasic nature of the elimination is provided. However, it should be remembered that the subcutaneous injection route of exposure is generally thought to result in slower release of compounds and may play a part in the kinetics.

4.3. METABOLISM

An early study of the disposition of orally administered resorcinol in rabbits was reported by Garton and Williams (1949). In this study, 65 % of the administered dose was recovered as glucuronide and sulfate conjugates (primarily the glucuronide) of the parent compound, and 11% was recovered as unchanged resorcinol within 24 hours of dosing. No evidence of "Phase I" oxidative metabolism was

1 Calculated as percent of the total delivered radioactivity, called "resorcinol equivalents" in Merker, et al. 1982.

detected.

Kim and Matthews (1987) investigated the fate of [^{14}C]-resorcinol after oral administration to male and female rats. Greater than 90% of the dose was recovered in urine within 24 hours. Approximately five percent of the administered dose was excreted as unchanged resorcinol. Urinalysis isolated four resorcinol metabolites, which were identified as glucuronide (the majority conjugate, by mass) and sulfate conjugates[2]. Repeated administration of resorcinol, 225 milligrams per kilogram body weight (mg/kg) once a day for five consecutive days, showed no changes in the rate or pattern of metabolism. This study was designed in part to detect sex differences in metabolism, but none were apparent.

Merker et al. (1982) found essentially similar metabolism of resorcinol using a different dosing route. As previously described, these investigators conducted a pharmacokinetic study after subcutaneous administration to rats (leading to longer delivery periods of the compound). In all cases, the major metabolite in urine was determined to be a glucuronide conjugate.

Studies of dermal exposure in humans (Yueng, et al. 1981; Yueng, et al. 1983) indicate the primary metabolite in excreta to be the glucuronide conjugate with lesser amounts of sulfate conjugates. No free resorcinol was detected in urine in these studies.

4.4. ELIMINATION

All studies previously cited indicate the primary pathway of elimination is urinary excretion as a water-soluble conjugate. Studies in animals suggest excretion of 76% (Garton and Williams, 1949) to greater than 90% (Kim and Matthews, 1987; Merker, et al, 1982) of total dose can be found in urine as conjugated metabolites within 24 hours of dosing. Similar urinary metabolites are observed in humans, although the elimination efficiency in humans has not been studied quantitatively.

5. HAZARD IDENTIFICATION

Effects following short-term (acute to subchronic) exposure to resorcinol have been studied in man and several of the observed actions have been reproduced in animal models. Both subchronic and lifetime bioassays of resorcinol have been conducted in laboratory animals. Findings are described below, with special detail on specific studies critical to the derivation of an oral RfD.

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2 Acid conjugation occurs at either or both hydroxyl moieties on the resorcinol molecule, leading to five possible

5.1. HUMAN

5.1.1. Acute

Industrial exposures to resorcinol have occurred in humans and the industrial hygiene literature suggests that high doses of the compound have actions analogous to a structurally similar chemical, phenol. The high-dose clinical signs include nervous system stimulation, followed by nervous system, cardiac, and respiratory depression. These effects have also been observed in patients treated with high topical doses of resorcinol (Bontemps, et al, 1995), usually where skin was not intact (e.g., treatment of leg ulcers). The general toxicology literature (e.g., Allan, 1994) identifies kidney, spleen, and liver involvement in cases of acute toxicity, but the information is tertiary (i.e., citation of reviews relying on yet previous citations) and primary citations for these effects are as much as 100 years old. The primary studies were not reviewed.

Poisoning reports in the clinical literature also indicate acute high doses in children produce methemoglobinemia and hemolytic anemia (Cunningham, 1956; Lundell and Nordman, 1973). Such effects have been seen following topical treatments to damaged skin (e.g., eczema).

5.1.2 Subchronic

The use of resorcinol ointments for chemical "peels" is evidence of the ability of the compound to irritate human skin at high concentrations. The industrial hygiene literature contains reports of dermatoses in workers using resorcinol-based adhesives (Abbate, et al. 1989). At concentrations more typical of mild therapeutic treatments, no irritation was seen in human volunteers (1.4%, or 14,000 mg/kg, in a cosmetic product; CIR, 1985[3]).

Resorcinol appears to be a sensitizer, producing allergic dermatitis in a proportion of individuals exposed repeatedly to the compound in cosmetic products (Eierman, et al. 1982).

It has been reported that humans using topical resorcinol products (as noted above, the compound is absorbed through the skin) for prolonged periods may experience interference with thyroid function, including goiter (Klein, et al. 1956; Thomas and Gisburn, 1961) and at least one author has suggested high incidence of goiter may be discernable in areas with elevated concentrations of

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permutations of glucuronic- (mono-, di-), sulfate- (mono-, di-), or mixed acids of resorcinol.

3 The Cosmetic Ingredient Review (CIR) is an expert panel of independent scientists who report on the safety assessment of cosmetic ingredients, including studies done by the industry but not published. Where AMEC relied on the description of a study by the CIR rather than the primary literature, it was cited as "CIR, 1985".

resorcinol and similar compounds in water supplies (Gaitan, 1988). It is not clear from the case reports whether these findings should be ascribed to subchronic or chronic exposure, although the pharmacokinetics of the compound (rapid elimination with minimal tissue distribution in animals) suggest that there may be little difference in delivered dose in subchronic versus chronic exposure situations. The effect has been reproduced upon acute dosing in animals (Arnott and Doniach, 1952; Samuel, 1955) and appears to be the result of interference by resorcinol in the normal synthesis of thyroid hormones. Interestingly, early attempts to produce the effect in animals using aqueous injections of resorcinol in rats failed. It was not until prolonged-release mechanisms of dosing (injection of oil-based solutions, topical application of ointments) were implemented that the thyrotoxic effects were observed (Samuel, 1955). This disparity arises as the result of the extremely rapid clearance of resorcinol – unless a method of maintaining adequate levels of the compound for prolonged periods is employed, interference with thyroid hormone synthesis is not interrupted for a sufficient period to be apparent as thyrotoxicity. Application of a 2% (20,000 milligrams per liter, mg/L) alcoholic solution of resorcinol twice a day, six days per week for four weeks produced no changes in thyroid hormone levels in human volunteers (n=3; Yeung, et al. 1983).

5.1.3 Chronic

No studies specifically relating to the effects of chronic exposure to resorcinol in humans were found.

5.2 ANIMAL

5.2.1 Acute

5.2.1.1 Acute lethality

A series of acute studies in animals are reported by Flickinger (1972). The median acute lethal dose (LD_{50}) for resorcinol by single oral administration to rats is 980 mg/kg (95% confidence intervals 740 to 1290 mg/kg). A study in rabbits indicated irritation to rabbit skin when pure resorcinol was "moistened" and placed under a patch for 24 hours. A dose-response relationship was reported for this experiment, but was reported as mass of resorcinol per kilogram body weight, so it is not possible to determine the relative concentration of resorcinol that was placed applied to the skin. The dermal LD_{50} derived from this experiment was 3360 mg/kg (95% confidence intervals 1980 to 5710 mg/kg). Flickinger (1976) also reported eye irritation using pure resorcinol in a rabbit eye test, but no pulmonary irritation (measured only by clinical observation) in rats exposed to as high as

2,800 milligrams of resorcinol per cubic meter (mg/m^3) as an aqueous aerosol for eight hours.

5.2.1.2 General Toxicology

The National Toxicology Program (NTP, 1992) performed a 17-day oral dosing study in rats and mice to support range finding for longer term studies of the compound. Groups of five rats of each sex were administered an aqueous resorcinol solution by gavage (stomach tube) in doses between 27.5, 55, 110, 225, and 450 mg/kg five days per week for a total of 12 doses over 17 days. A control group was administered deionized on the same schedule. Groups of five mice of each sex were dosed in a similar fashion, except the doses were 37.5, 75, 150, 300, and 600 mg/kg. Animals were observed for clinical toxicity, weighed, and survivors were killed and necropsied following treatment. Brain, heart, kidney, liver, lung, thymus were weighed (data is reported as absolute weight and organ-to-body weight ratio) and those taken from the highest dose group in rats and the two highest dose groups in mice were subjected to histopathology.

All rats survived the treatment, but resorcinol was lethal to mice at high doses. All females and four of five males died during treatment with 600 mg/kg. One male died at 300 mg/kg.

Clinical signs of toxicity were observed in males rats dosed at or above 225 mg/kg, while signs were observed in female rats at or above 55 mg/kg. Hyperexcitability occurred within one-half hour of dosing and lasted for one to two hours. Tachypnea (panting) was observed in male rats at or above 225 mg/kg and females administered doses of 110 mg/kg and 450 mg/kg. Tremors were observed in mice (males at or above 150 mg/kg; females at 300 and 600 mg/kg). Similar signs have been reported in rats dosed subcutaneously in a range finding study preceding pharmacokinetic analysis. (Merker, et al., 1982). These investigators observed the effect in animals given 140 mg/kg but not those administered 88 mg/kg. Due to the timing of the observations, NTP considered the toxic signs an acute central nervous system (CNS) effect of resorcinol.

Body weight and body weight gain was similar to controls in all treatment groups of both species. The only statistically significant change in organ weights occurred in the high dose female mouse group (thymus weight thymus-to-body weight ratio). No gross or microscopic lesions attributable to resorcinol were observed in any treatment group in either species.

A secondary review of earlier acute studies appears in a document prepared by the Cosmetic Ingredient Review (CIR, 1985). Rats were fed 5% (by weight) resorcinol in diet (estimated dose 260.7 mg/kg per day) for two weeks, or given two doses of 250 mg/kg by gavage, 24 hours apart. Clinical signs of the type described above were not reported in this study, but animals exposed by

the dietary route were found to have increased thyroid and decreased adrenal-to-body weight ratios (these tissues were not investigated in the 17-day study by NTP).

5.2.2 Subchronic

NTP undertook both subchronic and lifetime toxicity testing of resorcinol in rodents (NTP, 1992). In the 91-day studies, male and female rats and mice (10 animals per group) were given one of five doses of resorcinol by mouth. In mice ($B6C3F_1$ strain), the dose groups were 28, 56, 112, 225, and 420 mg/kg body weight per day (mg/kg-day), dissolved in water such that the daily dose was 10 ml/kg body weight. Dose groups in rats (Fisher 344/N strain) were 32, 65, 130, 260 and 520 mg/kg, dissolved in water such that the daily dose was 5 ml/kg body weight. Additional control groups for each species and sex were given an equivalent volume of water by gavage. Dosing was conducted 5 days per week for 13 weeks.

Animals were observed for clinical signs weekly. Necropsies were performed at the termination of the 91-day dosing period. Organ weights were recorded for the adrenal gland, brain, heart, kidney (right), liver, lungs, thymus, of all animals and testis (right) of the males, and histologic pathology (microscopic examination) of all organs were conducted on the two highest dose groups.

A summary of significant findings is provided in Table 1. Briefly, mortality was high in both species at the high-dose level. Liver-to-body weight ratios (not absolute liver weights) were increased in male mice given 28 to 112 mg/kg-day, but not in the group given 225 mg/kg-day (statistical evaluation of high-dose male mice was conducted and found not to be significant, however the test would be expected to have low power as there were only two survivors). Absolute liver weights and liver-to-body weight ratios were significantly higher in female rats administered resorcinol in doses of 65 mg/ kg-day or higher and male rats given 130 mg/kg-day or higher (the 520 mg/kg-day dose rate was not analyzed for either sex because there were few or no survivors). Liver-to-body weight ratios (not absolute liver weights) were significantly elevated in male rats given 32 or 65 mg/kg-day of resorcinol.

A confusing finding was a change in adrenal weights in male rats and mice in the 91-day study. While weights of this organ were <u>decreased</u> to a statistically-significant level in male mice (both absolute weight and organ-to-body weight ratio), a statistically significant <u>increase</u> was observed in male rats. Statistically-significant changes, albeit the opposite change, were observed at the lowest dose level (28 mg/kg-day in mice; 32 mg/kg-day in rats). Statistically significant increases were seen in adrenal-to-body weight ratio, but not absolute organ weight at the 225 mg/kg-day dose level in female mice, but not at lower doses. Interestingly, a decrease in adrenal weights of male rats is

reported in a study reviewed by CIR (1985).

Given the clinical findings in humans, an important negative finding in the NTP (1992) study was the lack of effect on the thyroid gland. No pathological change in this organ was observed in any treatment group. Thyroid hormone blood levels were measured in rats given 130 mg/kg-day resorcinol for 91-days and were found to be equivalent to measurements in control animals.

Clinically, the only observation reported was a transient qualitative effect of apparent CNS origin: ataxia (decreased movement), and the recumbency and tremors noted in the acute evaluation were seen in several dose groups (both species, both sexes). This observation has been reported in other animals studies (e.g., Merker, et al. 1982, NTP 17-day study) and was of concern to the Review Panel commissioned by the NTP. The finding typically occurred shortly after dosing and subsided within approximately one hour. This timing coincides with the rapid clearance of the compound and may indicate the effect is an acute response. CNS effects occurred only at the highest dose in the 91-day study (420 mg/kg-day and 520 mg/kg-day in mice and rats, respectively).

5.2.3 Chronic

Chronic toxicity studies of resorcinol reviewed by the CIR (1985) primarily relate to dermal application of the compound. However, the NTP undertook a chronic oral study and a report on these evaluations was released in 1992 (NTP, 1992). The 2-year studies had two dose groups, plus a control group for male rats and both sexes of mice (60 animals per group). Doses in this case were 112 and 225 mg/kg per day. Three dose groups, plus a control group of female rats (60 animals each) were used. The doses in female rats were 50, 100, and 150 mg/kg. Dosing was performed by gavage, 5 days per week for 103 weeks (animals were approximately 109 weeks old at the end of the study), using the same aqueous volumes as the 91-day study, described previously. The difference in dosing in female rats derives from high lethality encountered when the chronic study was initiated in this sex at 112 and 225 mg/kg. Several female rats died, necessitating a restart of the experiment at lower doses.

Animals were observed for clinical signs every 4 weeks. Necropsies were performed on 10 animals per group taken at 15 months from the 2-year study ("interim evaluation"). At the end of the 2-year studies, necropsies were performed on all remaining animals. Organ weights of the brain, kidney, and liver were recorded for the animals taken at interim evaluation. Histologic pathology was performed on all organs of (1) the mice taken at interim evaluation, (2) the control and high-dose male rats taken at interim evaluation, (3) remaining rats after the 2-year dosing period, and (4) control and high-dose male mice after the 2-year dosing.

A summary of significant findings in the 2-year studies is provided in Table 2. Briefly, mortality, measured as mean survival days, was significantly affected at the highest dose in male and female rats, but not mice. In contrast to the 91-day study, the only statistically significant effect on liver was an increase in liver-to-body weight ratio at the highest dose (150 mg/kg-day) in female rats. Adrenal weights were not recorded in the 2-year study by NTP, so further direct evaluation of the findings of organ weight change in the 91-day study was not possible. However, there were no statistically-significant differences relative to control in the incidence of neoplastic or non-neoplastic lesions of the adrenal gland noted in the histopathologic evaluation of chronically exposed animals.

Ataxia, recumbency, and tremors, previously observed in the 91-day study, were also noted qualitatively in several dose groups (both species, both sexes). The signs were observed at the lowest administered dose in mice and male rats (112 mg/kg-day) in the 2-year study. Female rats given 100 mg/kg-day in the 2-year study displayed the CNS effect, but it was not seen at 50 mg/kg-day.

No tumors were found to be present in dosed animals of either species or sex at incidences different from the control animals. This observation lead NTP to conclude that the study indicated no evidence of carcinogenic activity of resorcinol.

This finding is generally consistent with short- and long-term studies of mutagenesis and carcinogenesis by other investigators. Resorcinol has generally been negative in bacterial assays for mutagenicity (CIR, 1985), including those conducted by NTP preceding the chronic bioassay (NTP, 1992.) Tests in mammalian cells indicate the compound does not produce chromosomal aberrations or sister chromatid exchange (SCE, an exchange of sections of the DNA strand between chromatid pairs often observed in genetic material treated with mutagens) in Chinese Hamster Ovary cells. Human lymphocytes treated *in vitro* with resorcinol were negative for SCE, but did show chromosomal aberrations (Darroudi and Natarajan, 1983). *In vivo* treatment of rats with resorcinol (by oral, dermal, and intraperitoneal injection dosing routes) resulted in no SCE in cells harvested from the bone marrow after treatment (Bracher, et al. 1981).

Yamaguchi, et al. (1989) performed a promoting study on resorcinol. In a promoting study, a known carcinogen is administered and the effect of a second compound on tumor formation is studied. Promoting agents are defined as compounds that increase the tumor formation initiated by the carcinogen. Yamaguchi, et al. (1989) found that resorcinol promoted the effect of the carcinogen methyl-n-nitrosamine on stomach tumors in rats, but significantly reduced the formation of lung tumors.

Dermal application of resorcinol in mice and rabbits with a solution containing up to 50% resorcinol for 75 and 160 weeks, respectively, produced no dermal tumors in excess of control rates (Stenback and Shubik, 1974; Stenback, 1977). Burnett and Goldenthal (1988, see also IRDC, 1979, which contains the original data) performed a carcinogenicity study of several resorcinol-containing hair care formulations (hair dyes) by the dermal exposure route. One-half milliliter of the preparations containing 1.7 to 2% resorcinol by weight (other ingredients, including phenyl amines and various other hydroxyaromatic compounds, were also contained in these preparations) were applied to the backs of male and female rats twice per week for two years. Irritation, but no skin lesions were observed in animals taken as a 12-month interim sacrifice or at study termination. Tumor rates were compared to three separate controls groups and were only considered to be treatment related if the tumor rate in treated animals was significantly elevated relative to all three control groups. No treatment-related tumors were identified for resorcinol-containing preparations.

5.2.4 Reproductive and Teratology Studies

No oral multigeneration reproductive study of resorcinol was found in the literature. However, Burnett and Goldenthal (1988) evaluated several hair preparations applied dermally in a two-generation study. Preparations (one-half milliliter) were applied to the backs of 60 male and female rats twice weekly during growth, mating, gestation and lactation in the F_0 generation. This was more than 100 days of dosing and continued through weaning of F_{1a} animals (i.e., progeny of the first mating of the F_0 generation). A second mating of the F_0 generation was conducted with 20 matings of randomly selected males and females produced an F_{1b} group. Selected animals in the F_{1b} group were then treated in an equivalent fashion as the F_0 animals and bred to produce F_{2a} and F_{2b} offspring. Reproductive indices, including per cent pregnancies in each mated group, mean litter size, gestational index (live pups/total delivered pups), number of pups born dead, average number of pups weaned per litter, weaning index (number of pups surviving at day-21/number of pups retained in the litter at day-4), and sex-specific body weights at weaning in treated groups were compared to vehicle treated controls. There were no statistically significant differences between treated and control groups in any liter for any reproductive index. The dosing route for this study is not optimal for inference on an oral RfD for resorcinol, given both the nature of the treatment compounds (mixtures) and particularly because no mass-per-body weight dose was calculated. However, the authors express confidence that some systemic absorption occurred, based on previous studies of uptake (studies in the Absorption section of this proposal also indicate transdermal penetration) and thus it is reasonable to reach the qualitative conclusion that resorcinol is at least not a potent reproductive toxin.

Resorcinol has been tested for teratogenicity and the studies have been largely negative. While Kohonen, et al. (1983) saw weak effects of resorcinol on toxicity to chicken embryos, in more traditional teratogenicity assays, DiNardo, et al (1985) and studies conducted by Hazelton Laboratories[4], no teratogenic effects were observed in rats and rabbits given resorcinol by gavage. In the Hazelton rat study (Hazelton, 1982a), groups of 23 pregnant rats were given 40, 80, or 250 mg/kg of resorcinol in aqueous solution on days 6 through 15 of gestation (the critical organogenesis period) and maintained until gestational day 19, when the animals were killed and examined. Negative vehicle control and positive (Vitamin A 15 mg/kg) groups of equivalent size were also treated and examined during the study. Ovaries and uteri were collected and corpora lutea were counted. Implantations were counted and classified as (a) live fetuses, (b) early intrauterine deaths, (c) early/late intrauterine deaths and (d) late uterine deaths. Fetal weights were observed, fetuses were sexed, and half were stained with alizarin red for evaluation of skeletal abnormalities. The remaining fetuses were fixed with Bouin's solution for examination of visceral abnormalities.

All resorcinol-treated dams survived and no clinical signs of toxicity were observed during the study. Mean body weight of dams in the high-dose group was lower than other groups but not statistically significant. There was no effect of resorcinol on implantation. Early intrauterine loss was higher in all resorcinol-treated and was statistically significant by chi-square tests. However, the loss rate was very low (0.4 to 0.5 deaths per dam versus 0.3 deaths per dam in controls) and did not result in an overall post-implantation loss rate that was statistically different from the negative control. Post-implantation loss was not dose-correlated (2.9% loss in controls versus 4.1, 7.2 and 3.8% loss in animals treated with 40, 80, and 250 mg/kg resorcinol, respectively). The authors thus did not interpret the findings as a true treatment effect. There were no differences in sex ratios of the litters among treatment groups, no external malformations in excess of control rats, and no visceral (organ) or skeletal malformations. Skeletal "variations" (defined as changes that occur frequently even in control groups and without functional significance), primarily incomplete ossification of the parietal, interparietal, or occipital bones of the skull and extra ribs, were higher in treated groups, but not statistically different from controls (2%, 7.7%, 8.5%, and 10.6% of fetuses had skeletal variations in negative control, and 40, 80, and 250 mg/kg resorcinol, respectively). Extremely significant increases in the incidence of skeletal variation (89%) were observed in the positive control. The overall conclusions of this study were that oral exposure to resorcinol up to 250 mg/kg was not embryotoxic, embryolethal, or teratogenic in rats.

The findings of the Hazelton rat study are concordant with the results of DiNardo, et al (1985), who

4 Hazelton studies were not published in the general literature, but were obtained by AMEC from the CIR

studied Sprague Dawley rats (10 to 13 per group) given resorcinol 125, 250, and 500 mg/kg-day resorcinol by gavage on days 6 through 16 of gestation. Animals were killed on gestation day 20 and uteri were examined for implantations, presence of live and dead fetuses, and sex ratios. All fetuses were examined for external abnormalities and half were fixed in Bouin's solution for visceral exam. The remaining fetuses were fixed with alizarin red for examination of skeletal abnormalities. DiNardo (1985) does not present the raw data for the study, but indicates that no study endpoint for treated animals was statistically different from controls. The conclusion was that resorcinol up to 500 mg/kg was not teratogenic in rats.

In the Hazelton rabbit study (Hazelton, 1982b), groups of between 20 and 26 New Zealand white rabbits were given 25, 50, or 100 mg/kg of resorcinol in aqueous solution on days 6 through 18 of gestation (the critical organogenesis period) and maintained until gestational day 28, when the animals were when the animals were killed and examined. Negative vehicle control and positive (Vitamin A 15 mg/kg) groups of equivalent size were also treated and examined during the study. Evaluations were identical to those described for rats.

Weight gain was similar to controls in low and intermediate dose groups, but lower in the high dose group. Several animals died in both the treatment and control groups, but with the exception of a spontaneous abortion in the 50 mg/day group, all other deaths were associated with either pneumonia or intubation errors.

There was no effect of resorcinol on pregnancy rates or implantations and sex ratios were similar in all groups. Two does in each of the control and 25 mg/kg group (out of 16 and 13 pregnant animals, respectively) were found to have total intrauterine death. Post-implantation losses discounting these animals were found to be higher in the 25 and 50 mg/kg groups but not the high dose group. Including the animals with total intrauterine death results in post implantation losses that were higher than controls only in the 25 mg/kg group. No differences were statistically significant and all were interpreted by the authors to be within the typical range for post implantation loss observed in historical controls. Thus, no treatment-related effect was inferred. No external, visceral or skeletal malformations were observed in resorcinol-treated or negative control groups. Skeletal variations, which were primarily ossification effects on the sternum and extra ribs, were observed to be higher in the intermediate and high resorcinol dose groups. However, the variation from control rates were low (80, 85.7, 88.2 and 84.3% fetuses had skeletal variations in vehicle, 25, 50, and 100 mg/kg resorcinol groups, respectively) and not statistically significant. The overall conclusions of this study were that oral exposure to resorcinol up to 100 mg/kg was not embryotoxic, embryolethal, or teratogenic in rabbits.

6. DOSE RESPONSE

Section 250.605 paragraph (b), of the PADEP regulations indicates that, in the absence of toxicity factors from cited sources, a person may, subject to approval of the Department, develop standards from literature toxicity information on a compound by applying EPA guidelines on the determination of toxicity factors.

EPA (1993) and others (e.g., Dourson and Stara, 1983; Beck, et al 1994) describe a method for establishing toxicity factors (RfDs) for non-carcinogenic compounds by applying "uncertainty factors" to the highest dose level found to be without observable adverse effects (the No Observed Adverse Effect Level; NOAEL) or in some cases the Low Observed Adverse Effect Level. (LOAEL). Thus, an RfD is computed as follows:

$$RfD = \frac{\text{LOAEL or NOAEL}}{\text{UF x MF}}$$

Where UF is the product of a series of individual factors accounting for various sources of uncertainty and MF is a modifying factor used to account for uncertainty not specifically addressed by the standard uncertainty factors. Uncertainty factors are usually 10-fold increments and account for (1) extrapolation from animals to humans (UF_A) and (2) potential sensitive members of a population (UF_H). Under the EPA system (Dourson, 1994), additional uncertainty factors are considered to convert lowest Observed Adverse Effects Levels (LOAEL) to a NOAEL (UF_L), extrapolate from subchronic to chronic experiments (UF_S), and to account for the completeness of the body of toxicological studies on the compound (UF_D).

More recently, scientists and the World Health Organization International Programme on Chemical Safety have suggested certain factors could be divided into partial factors to separate pharmacokinetic (absorption, distribution, metabolism) from pharmacodynamic (mechanism of action) explanations for differences between species or individuals (IPCS, 1994; Renwick 1993; Silverman, et al. 1999). IPCS (1994) has suggested that the species extrapolation be apportioned equally between pharmacokinetic and pharmacodynamic considerations. That is, a factor of 3.2 could be used for each, which is a "half-log" of 10 and reflects the multiplicative nature of uncertainty factors. Under the EPA system, a similar "fractionation" of the traditional uncertainty factors has been established (EPA, 1994, in the case of EPA the factor has been rounded to three) and has been applied in many of the derivations archived in the IRIS database.

The following sections identify the critical effect and dose from the resorcinol literature that are

proposed for application to the EPA methodology and justify appropriate values for each of the EPA uncertainty factor categories.

6.1. CHOICE OF CRITICAL STUDY AND EFFECT

Acute dose studies are inappropriate for derivation of chronic RfDs, although they may provide ancillary information important to the selection of uncertainty factors and identify potential target organs and organ system effects. Teratology and Reproductive studies of resorcinol have been negative, while other toxicological endpoints have been observed at lower doses in subchronic and chronic exposure studies. The subchronic and chronic studies conducted by NTP are the most comprehensive in terms of tested toxic endpoints and utilized an exposure route (oral) most applicable to the information needed for evaluation of environmental exposure. Accordingly, it was concluded that the NTP (1992) studies were most appropriate for identifying doses that could be adjusted to infer a toxicity factor.

Observed effects in the subchronic study were mortality, increased liver weights, changes in adrenal weights, and qualitative CNS signs. Mortality and qualitative CNS signs were observed in the chronic study.

The increased liver weights were observed only in the subchronic study and were not reproduced in the chronic study. This casts doubt on this endpoint as a significant or adverse effect. Similarly, the finding of adrenal gland weight changes in animals treated subchronically was confusing in that the observed changes were the opposite in different species: adrenal weights increased in rats and decreased in mice. The effect cannot be evaluated directly using the results of the chronic study, as the glands were not weighed at necropsy in this study. However, there were no adverse clinical, hematological, or histopathological signs (including specific histopathology of the adrenal gland) in the chronically-treated animals that indicate adrenal weight change, if it were present during chronic dosing, was adverse to these animals. Likewise, no physiological indices were found in the subchronic study to indicate adrenal gland impairment. As such, the biological significance of the effect is questionable and it was not selected as the critical effect for computation of an RfD.

CNS signs were a qualitative clinical observation and, as is noted in the NTP report, are therefore difficult to assess in terms of dose-responsiveness. The finding was not quantified to the level that it could have been statistically analyzed and levels of severity and their correlation to dose were not recorded or analyzed. Thus, this effect was not selected as the critical effect for RfD derivation. However, the observation indicates that a data gap in the toxicological information on resorcinol exists, since no neurotoxicological battery on the compound appears to have been conducted. As

will be described, this was considered in establishing an uncertainty factor related to data quality and completeness.

Mortality was selected as the critical effect. It is a clear cut endpoint for resorcinol in chronic dosing studies in rats and, indeed, the NTP (1992) report noted that dosing in female rats had to be reduced due to high mortality at the outset of the initial experiment. Mortality was rigorously evaluated statistically and the results were clearly dose related. Thus, mortality is proposed as the appropriate critical endpoint for the derivation of an RfD for resorcinol.

Mortality, measured as mean survival days by NTP (1992), was reduced to a statistically-significant degree at the highest dose in chronic rat experiments (225 mg/kg-day in males and 150 mg/kg-day in females) and was considered to be a frank effect level. The next lower doses (112 mg/kg-day in males and 100 mg/kg-day in females) were not different from controls (or yet lower doses, in the case of female rats) and were thus NOAELs. The lower of the sex-specific NOAELs, 100 mg/kg-day, is proposed as the critical effect level for calculating an RfD. As this dose level was administered five of every seven days, the daily dose is adjusted to 100 x 5/7 $\approx$ 71 mg/kg-day.

6.2. CHOICE OF UNCERTAINTY AND MODIFYING FACTORS

Specification and justification of a value for each of the five uncertainty factors described above are provided in this section.

6.2.1 Animal to Human (Interspecies) Extrapolation (UF_A)

While studies of resorcinol have been conducted in man, they were primarily acute low-dose experiments for the purposes of obtaining pharmacokinetic data. The critical study used for the RfD was conducted in rats and mice and has no close counterpart in a human study. The similarity of pharmacokinetic data between experimental animals and humans could argue for a smaller extrapolation factor. However, the pharmacokinetic data suggesting extremely rapid clearance of the compound after "bolus" doses (i.e., a dose given all at once over a short period of time) also suggests that the stomach tube delivery used in the NTP study might not be optimal for characterization of human exposure that could occur repeatedly over the course of a day (e.g., multiple drinks of water, bathing, domestic use of water). As such, it was concluded that a UF_A of 10 was appropriate.

6.2.2 Human Sensitivity (Intraspecies) Extrapolation (UF_H)

There are no studies identifying the range of sensitivities to resorcinol among humans. Accordingly, the default UF_H of 10 was considered appropriate.

6.2.3 LOAEL to NOAEL Extrapolation (UF_L)

A dose for which the critical effect is absent was available from the study data and has been interpreted as a NOAEL. Therefore the UF_L was set at one.

6.2.4 Subchronic to Chronic Extrapolation (UF_S)

The critical study used for the RfD was conducted for a majority of the typical lifetime for rats. Therefore no extrapolation from subchronic data were required and the UF_S was set at one.

6.2.5 Data Base (UF_D)

The critical study was conducted in a large number of animals and included a large number of general toxicology endpoints. The results were largely consistent both among studies conducted as part of the NTP resorcinol study program, as well as similar studies conducted by independent investigators. Multiple species were evaluated in the NTP studies and by other investigators. Several teratology studies have been conducted in multiple species and have demonstrated that resorcinol is not teratogenic. Pharmacokinetic studies have been conducted in both laboratory animals and humans and suggest extremely similar handling of the compound between the species.

Data gaps do exist in the toxicological information on resorcinol. First, while a multigenerational reproductive study has been conducted with resorcinol, it was conducted using dermal application of the compound. It is thus not possible to determine if the doses were sufficiently high or applicable to the oral exposure route to have confidence in the negative findings. Second, qualitative observations of CNS signs in many experiments, while not adequate to comprise a critical endpoint, indicate that a neurotoxicological battery may be an important evaluation for resorcinol.

In summary, a strong body of general toxicology studies on resorcinol exists, but has data gaps for specific endpoints. These disparate features of the information suggest that a UF_D greater than one should be applied but may mitigate against the requirement for application of a full 10-fold default value. A UF_D of 10 was applied to account for incomplete study of neurotoxicity and uncertainty concerning the adequacy of reproductive studies, but is considered quite conservative.

6.2.6 Modifying Factor

A modifying factor of one was selected, as no additional adjustment for uncertainty was considered necessary.

6.3 CALCULATION OF THE REFERENCE DOSE

The RfD is computed by dividing the NOAEL by the product of selected uncertainty factors. For resorcinol, the calculation is:

$$RfD = \frac{71 mg/kg-day}{10 \times 10 \times 10 \times 1 \times 1 \times 1} = 0.07 mg/kg-day$$

Where 71 mg/kg-day is the NOAEL for mortality; a 10-fold value each is applied for UF_A, UF_H, and UF_D, respectively; and UF_L, UF_S and the modifying factor are all assigned a value of one.

6.4 CONFIDENCE

The critical study supporting the RfD was large and well conducted. As such, confidence in this study is high. The overall database on toxicological effects of resorcinol including human studies is extensive, but does appear to have data gaps relating to neurotoxicity study as well as questions as to the adequacy of the reproductive studies. Therefore the confidence in the database as a whole is medium, using U.S. EPA terminology noted in the Integrated Risk Information Service (IRIS, 2001). Using the same narrative approach, overall confidence in the RfD is also medium

It is of some interest to consider the main data gap for resorcinol, the absence of a neurotoxicology battery. If one were to accept the CNS effects observed in the chronic rat study could be treated as a quantal effect, a NOAEL could be established at the lowest dose in female rats (50 mg/kg-day 5 days per week, adjusted to a daily dose of 36 mg/kg-day), where no CNS signs were encountered. The uncertainty factors described above, would still apply, with the exception that it would be justifiable to use less than the default value for UF_D. That is, the lack of neurotoxicity information was the primary reason for using a 10 in this category and could be replaced by the assumption that the resorcinol is indeed neurotoxic. The quality of the database is still not optimal, given questionable adequacy of the reproductive study, but is largely complete and the data are of high quality, which argues for use of a 3-fold uncertainty factor. Dividing 36 mg/kg-day by the product of uncertainty factors (300) yields a higher than RfD than that calculated for the mortality endpoint above:

$$RfD = \frac{36\,mg/kg-day}{10 \times 10 \times 3 \times 1 \times 1 \times 1} = 0.12\,mg/kg-day$$

Thus, this data gap is unlikely to affect overall conclusions concerning the appropriate RfD.

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