

**Description of a Proposed Reference Dose
Resorcinol**

prepared for review by

**The Resorcinol Peer Review Panel
Toxicology Excellence for Risk Assessment**

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EXECUTIVE SUMMARY

This paper reviews toxicological studies of resorcinol with the purpose of deriving a Reference Dose (RfD). Resorcinol is the common name for m-dihydroxybenzene (CAS # 108-46-3; also known as 1,3-benzenediol or m-hydroxyphenol).

Key studies, critical effect(s) and associated doses (No Observed Adverse Effect Levels and/or Lowest Observed Adverse Effect Levels, NOAELs; LOAELs) are identified in the report and adjusted using uncertainty factors to determine an oral RfD. This report proposes the number and magnitude of uncertainty factors to be used in the computation.

ES-1 Pharmacokinetics

The results of pharmacokinetic studies in rats, rabbits, and humans 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, pharmacodynamics notwithstanding, animal toxicity testing of resorcinol should provide good information on potential effects in humans. In addition, pharmacodynamic comparisons between rodents and humans have demonstrated that rodents are much more sensitive to the effects of thyroid disrupters than are humans.

ES-2 Hazard Identification

Effects following short-term (acute to subchronic) exposure to resorcinol have been studied in humans, 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. Symptoms described after acute high dose exposure in humans include nervous system stimulation, followed by nervous system, cardiac, and respiratory depression, and hematological abnormalities.

Subchronic exposures have demonstrated that resorcinol has thyrotoxic effects in both humans and animals when high doses are maintained. Resorcinol is also reported to produce irritation and allergic dermatitis in a small proportion of individuals.

Acute studies of resorcinol toxicity in animals have determined a median acute lethal dose (LD50) of 980 mg/kg in rats after oral administration and a rabbit dermal 24 hour LD50 of 3360 mg/kg.

In sub-acute testing, resorcinol was administered to rats and mice by oral gavage for 12 of 17 days. All rats survived the treatment of up to 450 mg/kg body weight per day (mg/kg-day). Ninety percent of mice survived administration of resorcinol at 300 mg/kg-day, but 600 mg/kg-day was lethal to ninety percent of mice. Effects observed after resorcinol administration included transient central nervous system (CNS) stimulation in both rats (hyperexcitability, tachypnea) and mice (tremors). No gross or microscopic lesions attributable to resorcinol were observed in any treatment group in either species. In another sub-acute study, rats given approximately 261 mg/kg-day in the diet for 4 weeks had no apparent CNS effects but had decreased adrenal-to-body weight ratios. Short-term studies involving administration of resorcinol to rats in the food (5 per cent in the diet for two weeks, which is equivalent to 4,000 mg/kg-day) or water (10 mg/kg-day for 30 days) resulted in increases in thyroid weight. Changes in circulating thyroid hormone levels were observed in the feeding study, while reduction of iodination of thyroid hormones was reported in the drinking water study. Rats given 40 mg resorcinol per liter of drinking water for 90 days (equivalent to 6 mg/kg-day) had morphometric changes in thyroid cells.

In a subchronic study, resorcinol was orally administered to rats and mice by gavage at doses up to 420 mg/kg-day (mice) or 520 mg/kg (rats), 5 days per week for 13 weeks. CNS effects occurred only at the highest dosages. Mortality was high in both species at the high-dose level. Liver-to-body weight ratios were increased. Adrenal weight changes were observed in both rats and mice, while kidney-to-body weight ratios and brain-to-body weight ratios were elevated in mice and male mice only, respectively. In this study, resorcinol administration did not produce any thyroid effects in either rats or mice.

In a recent, subchronic dose range finding study performed by WIL Research Laboratories and sponsored by the Resorcinol Task Force (RTF), male and female CrI:CD(SD)IGSBR rats were exposed to resorcinol in drinking water at doses up to 360 mg/L for up to 87 days (WIL, 2003). The objective of this study was to identify doses of resorcinol that would cause minimal toxicity and yet allow for successful mating, gestation, parturition and lactation. This dose range finding study was performed as a precursor to a guideline compliant two-generation reproductive study. Survival, mean body weights, mean organ weights, and reproductive performance were not affected. Among the various sensory and behavioral tests conducted, no statistically significant effects were observed that could be attributed to resorcinol. No effects were reported on the thyroid. Minimal microscopic changes of the thyroid (i.e., follicular cell hyperplasia) were reported in both males and females. The authors reported that none of the differences in the incidence of follicular hyperplasia were statistically significantly different from controls. AMEC has performed additional statistical tests by merging the animals in the male interim sacrifice group, the male scheduled sacrifice group and the female scheduled sacrifice group and has found that incidence of follicular hyperplasia of the thyroid was statistically significantly different from controls when animal groups were merged for statistical significance testing purposes. Thus, the high dose level could be considered either a NOAEL or a LOAEL depending on the statistical approach followed. However, there is uncertainty associated with merging data from male and female animals because of the potential gender specificity of resorcinol's effects on the thyroid, and AMEC believes that others reviewing the same data would conclude that 360 mg/L is a NOAEL for thyrotoxicity.

The National Toxicology Program (NTP) conducted two-year studies in rats and mice administered resorcinol by the oral route by gavage at dosages of up to 225 mg/kg-day. CNS effects were observed in both species. Mean survival was significantly affected at the highest dose in male and female rats, but not mice. The only other statistically significant effect was an increase in liver-to-body weight ratio at the highest dose (150 mg/kg-day) in the female rats.

No evidence of carcinogenic activity of resorcinol was observed in the NTP two-year study. Additional experiments on several species (mice, rabbits, rats) have demonstrated negative results for tumorigenicity after chronic dermal application of resorcinol or resorcinol-containing products. Resorcinol has generally been negative in bacterial assays for mutagenicity. *In vitro* tests in mammalian cells are mixed. Resorcinol does not produce sister chromatid exchange

(SCE) in hamster V79 cells or human lymphocytes. Human lymphocytes treated *in vitro* with resorcinol were negative for SCE but did show chromosomal aberrations, and SCE is observed in resorcinol-treated Chinese hamster ovary cells. *In vivo* treatment with resorcinol has generally had negative results in chromosomal assays. For instance, 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.

Teratogenicity tests have been conducted with resorcinol in both rats and rabbits. These studies demonstrated that oral exposure to resorcinol was not embryotoxic, embryolethal, or teratogenic at doses of 250 and 500 mg/kg in rats, or 100 mg/kg in rabbits.

ES-3 Dose-Response

For the purposes of this report, the critical effect for resorcinol has been defined as adverse effects on the thyroid. Reasons for defining thyrotoxicity as the critical effect are as follows: Thyrotoxicity has been observed in humans using resorcinol-containing pharmaceuticals at high doses for extended periods of time, and *in vitro* studies show that resorcinol can reversibly affect thyroid function. The impact of resorcinol on thyroid function has been discussed in a recent document issued by the European Commission entitled *Study on the Scientific Evaluation of 12 Substances in the Context of Endocrine Disrupter Priority List of Actions*. This 2002 document was prepared by the consulting company WRc-NSF and specifically focused on chemicals that previous literature had targeted as potential endocrine disrupters. Previous European Commission documents had identified resorcinol for study due to its reported thyroid effects. The WRc-NSF document summarized the reported toxicology of resorcinol as follows:

In vitro studies indicate that the anti-thyroidal activity observed following resorcinol exposure is due to inhibition of thyroid peroxidase (TPO) enzymes, as evidenced by disruption of thyroid hormone synthesis and changes in the thyroid gland consistent with goitrogenesis.

Certain older *in vivo* laboratory animal studies have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high doses and required a vehicle (such as peanut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable.

Studies conducted as part of the National Toxicology Programme have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight⁻¹ day⁻¹ in rats and

450 mg kg body weight⁻¹ day⁻¹ in mice for 13 weeks and 150 – 225 mg kg body weight⁻¹ day⁻¹ for 5 days per week over 2 years in rats and mice.

There is evidence of effects on adrenal weights at all doses tested in NTP rat and mouse 13-week studies. However, the observed responses did not show dose-dependent relationships.

Currently available data indicate that resorcinol is not embryotoxic or teratogenic.

Other scientists and panels of experts who have reviewed the available toxicology information on resorcinol have reached similar conclusions. Many of these literature summaries are discussed in this RfD derivation document.

Given that thyrotoxicity is the critical effect for resorcinol, there are a limited number of candidate studies to consider for the key study. Human case studies of individuals who received extremely high doses of resorcinol are inappropriate for RfD derivation. No properly designed and executed human epidemiology studies exist that are suitable for RfD derivation. Two subchronic drinking water studies in rodents show adverse effects on the thyroid, but both of these studies were classified as “use with care” by the European Commission summary on resorcinol.

The first of the two studies (Seffner et al., 1995) was not as useful as the WIL (2003) data for RfD derivation for several reasons: (1) no dose information was presented, (2) only a single drinking water concentration was tested, (3) no measurement of overt pathology or thyroid hormone levels was evaluated in this study, and (4) only changes in the size of thyroid cells were measured. The second (Cooksey et al., 1985) was not as useful as the WIL (2003) data because: (1) no information was provided on the concentration of resorcinol in water, (2) only a single drinking water concentration was tested, (3) thyroid weights were presented only graphically, and (4) no measurement of overt pathology or thyroid hormone levels was evaluated in this study, only measurement of incorporation of radioiodine after a 3-hour bolus dose.

Although no thyroid effects were seen in any animal group, the NTP subchronic and chronic studies also were not as useful as the WIL (2003) data for RfD derivation for three reasons: (1) dosing was gavage bolus and not continuous, which would be more relevant for RfD

development, (2) circulating thyroid hormone levels were only measured for male and female rats in the 13-week study and (3) only T₃ and thyroxine were measured, not TSH.

The subchronic drinking water study in rats performed by the WIL Laboratories for the Resorcinol Task Force (RTF) was selected as the key study for derivation of an RfD because it is a fully documented study that measured multiple endpoints, including thyroid weight, circulating T₃, thyroxine and TSH hormone levels, and thyroid histopathology. Thyrotoxicity, i.e., follicular cell hyperplasia, was selected as the critical endpoint for the derivation of an RfD for resorcinol. The thyroid hyperplasia was not accompanied by thyroid hypertrophy or changes in circulating thyroid hormones. The prevailing model for the mode of action for thyrotoxic chemicals is that alterations in circulating thyroid hormones (decreases in thyroxine and T₃, increases in TSH) are early biological effects that precede altered thyroid structure, which includes thyroid cell hypertrophy followed by hyperplasia (Capen, 1997, 1998, 2000; Hard, 1998). No adverse effects on thyroid function were demonstrated. The observed hyperplasia could have been artifactual, given the fact that no differences were observed in circulating thyroid hormones compared to controls.

The combined male and female high dose level of 47.7 mg/kg-day is proposed as the critical effect level or Point of Departure (POD) for calculating an RfD. This dose level is a No Observed Adverse Effect Level (NOAEL) based on statistical significance testing of each animal group separately. When statistical tests are performed by merging into a single group the animals in the male interim sacrifice group, the male scheduled sacrifice group and the female scheduled sacrifice group, this dose can be considered a Lowest Observed Adverse Effect Levels (LOAEL). However, as noted previously, this approach is subject to uncertainty because of the potential gender specificity of resorcinol's effects on the thyroid.

When 47.7 mg/kg-day is considered the NOAEL for thyroid toxicity, three-fold uncertainty factors are appropriate for: (1) the potential presence of sensitive members of a population (UF_H), (2) extrapolation from subchronic to chronic study duration (UF_S), and (3) to account for the completeness of the body of toxicological studies on the compound (UF_D). A one-fold factor is appropriate for extrapolation from animals to humans (UF_A) and (2) extrapolation from a LOAEL to NOAEL (UF_L). Thus, for resorcinol the estimated RfD is approximately 2 mg/kg-day (47.7/30).

Alternatively, when 47.7 mg/kg-day is considered the LOAEL for thyroid toxicity, three-fold uncertainty factors are appropriate for: (1) the potential presence of sensitive members of a population (UF_H), (2) extrapolation from subchronic to chronic study duration (UF_S), (3) extrapolation from a LOAEL to NOAEL (UF_L), and (4) to account for the completeness of the body of toxicological studies on the compound (UF_D). A one-fold factor is appropriate for extrapolation from animals to humans (UF_A). Thus, for resorcinol the estimated RfD is approximately 0.5 mg/kg-day (47.7/100).

ES-4 Uncertainty

The key study supporting the RfD evaluated many different endpoints including specific endpoints of interest, but it involved a limited number of animals, being a dose range finding study with a specific objective. Its objective was to identify doses of resorcinol that would cause minimal toxicity and yet allow for successful mating, gestation, parturition and lactation in a full two-generation reproductive study. As such, confidence in this study is medium. The overall database on toxicological effects of resorcinol including human studies is extensive and includes subchronic and chronic National Toxicology Program rat and mouse bioassays, but it does appear to have a data gap relating 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 System (IRIS, 2004). Also, there is uncertainty associated with merging data from male and female animals when performing statistical significance testing on thyrotoxicity data, because of the potential gender specificity of resorcinol's effects on the thyroid. Using the same narrative approach, overall confidence in the RfD is also medium. The uncertainty factors applied to derive the RfD are reflective of the medium confidence in the RfD database, such that the level of 0.5 to 2 mg/kg-day adequately meets the general definition of the RfD given in Section 1 and represents a dose without appreciable human risk of adverse effect for daily exposure over chronic exposure periods.

Based on its review of the available database, AMEC has concluded that there is adequate toxicity information available to support the derivation of a RfD at this time. However, AMEC acknowledges that the key study, while superior to other existing studies for RfD derivation, is a dose range finding study that was performed as a precursor to a guideline compliant two-

generation reproductive study. In the future, when the full guideline compliant two-generation reproductive study has been completed, fully documented, and released to the public, AMEC may revise its RfD derivation to take into account the new information. The addition of the results of the full two-generation reproductive study using drinking water as the route of administration to the toxicological database will likely change the confidence in the database from medium to high.

1. INTRODUCTION

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) for resorcinol. 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], 2004)

Fundamentally, the approach to the derivation is to obtain information on the physical chemistry, pharmacokinetics, and toxicology of the compound in question and, equipped with that information, to make judgments on what dose of the compound would meet the definition cited above. The implicit assumption of the method is that the toxicity of most chemicals is monotonically dose-responsive. That is, if an effect is observed at some dose, the probability of the effect will be less, or even zero, at lower doses. For no effect levels, observable effects will not appear at yet lower doses. This paper concerns itself with an appropriate RfD for oral exposure.

The information required to establish an RfD may be obtained from the literature or generated *de novo*. In the case of resorcinol, a substantial amount of information has been presented in peer-reviewed journals or published as publicly available laboratory reports.

The authors of this report were aware at the outset of this investigation of two comprehensive reports on resorcinol: a safety assessment conducted by the Cosmetic Ingredient Review (CIR,

1985) and a report on a lifetime rodent bioassay of resorcinol by the National Toxicology Program (NTP, 1992). These reports and their bibliographies were used to initiate the information search on resorcinol. The CIR (1985) identified several unpublished toxicology testing reports, which were obtained from the CIR document service. Authors of important papers identified in the two cited references were “searched forward” using the National Library of Medicine PubMed database system and a general search on this system was also conducted using “resorcinol toxicity” as key words. Recently, two reviews of the thyrotoxic properties of resorcinol were published (Lynch et al., 2002; Wrc-NSF, 2002). These monographs and their bibliographies also proved to be useful for identifying pertinent information.

In addition to the targeted literature searching discussed above, a comprehensive *de novo* literature search was conducted utilizing PubMed, the National Library of Medicine's database search engine. All citations in the PubMed database published since 1985 that contained the word resorcinol or the resorcinol chemical abstract service registration number (CASRN 108-46-3) in any field were downloaded for review.

Five hundred and eighty-eight citations were downloaded. Abstracts were reviewed for any content relevant to resorcinol RfD development and classified by the context in which resorcinol was involved. Seventy-four of the downloaded citations contained information on resorcinol in the context of human or animal exposures, or were determined to provide insight to the potential mechanisms of resorcinol-induced effects in humans or animals. Of these 74 citations, AMEC had already reviewed 20. Of the 54 new articles identified, 44 were obtained and reviewed. The remaining 10 articles were not reasonably available from Boston-area libraries or on-line literature retrieval services. However, the abstracts did not indicate that they contained any new information useful for RfD development. Nine of the 10 citations contained information on allergic sensitization from the dermatological use of resorcinol, and one case study had information on resorcinol-induced methemoglobinemia.

The remaining 514 resorcinol citations in PubMed did not contain toxicity information useful for RfD development (Table 1). One hundred and eighteen papers involved a compound in which the resorcinol structure was a moiety of a much larger molecule, for example 4-(2-pyridylazo) resorcinol. One hundred twenty-six citations covered the use of resorcinol as a component of a surgical and dental adhesive formula; 52 citations involved the use of resorcinol as a reagent for

the detection of sialic acids for the quantitation of glycolipids; 18 citations discussed resorcinol as a component of Weigert's resorcin-fuchsin reagent, a histological dye used for the visualization of elastin; one discussed the inhibitory effects of catechol derivatives on arachidonic acid-induced aggregation of rabbit platelets; and one study discussed tumor promoters for nitroso-compound induced cancers in rodents.

Another 47 citations were analytical method papers that described how resorcinol is used as a substrate to assess microbial degradation potential (30 citations), or methods used for the separation and/or detection of resorcinol itself (17 citations). Twenty-eight papers used resorcinol in the investigation of chemical reactions, for example as a starting chemical in the synthesis of more complex molecules, or the interaction of resorcinol with other chemicals, e.g., polymerization reactions. Seventeen citations discussed the use of resorcinol in *in vitro* biochemical investigations for its ability to attract divalent metals. Sixteen citations discussed the use of resorcinol for various other *in vitro* effects, including effects on leucotriene and prostaglandin synthesis, glycogen synthesis, and glutathione levels. Thirteen citations discussed how resorcinol is a substrate for peroxidase enzymes and its use in *in vitro* investigations as a peroxidase inhibitor. Eleven citations involved the investigation of the antioxidant properties of resorcinol and other redox cycling phenolic compounds including the ability to scavenge reactive oxygen species. Eleven citations involved resorcinol as a metabolite of phenol, 3-dinitrobenzene or other more complex molecules. Similarly, 13 citations discussed the use of resorcinol as a substrate for the *in vitro* investigation of hydroxylase activity.

Five abstracts described natural occurrences of resorcinol including resorcinol in humic substances, in sorghum plants, and in cigarette smoke. For 22 of the downloaded abstracts, it was clear that no toxicological information for resorcinol would be presented in the paper. Fifteen papers published in foreign languages (Russian, Chinese, Japanese, Polish, Greek, Hungarian, Romanian and Portuguese) were evaluated by English abstract or title. All were found to have no value relevant to RfD derivation.

A similar search was conducted on the TOXLINE® database (<http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE>), the National Library of Medicine's (NLM) bibliographic database for toxicology. The five hundred and seventy-two citations that contained the word resorcinol or

resorcinol's CASRN published since 1985 were downloaded. Many of the articles deemed to be appropriate for RfD derivation were already in AMEC's possession, having been identified in recent summary publications or in the PubMed search previously discussed. As with the PubMed search discussed above, however, most of the articles identified by TOXLINE® were determined by an evaluation of the title and abstract to be irrelevant to RfD derivation. The TOXLINE® search did identify 14 potentially relevant articles that AMEC had not reviewed after consideration of the results of the PubMed search. Eight of these articles were obtained and reviewed by AMEC, the remaining six citations were not reasonably available, because they were not articles published in scientific journals.

Of the six potentially relevant citations not reasonably available, three were works by Eduardo Gaitan, two of which were meeting proceedings. AMEC has reviewed nine other papers by Mr. Gaitan and has evaluated many summary publications that discuss this work. AMEC has determined that Mr. Gaitan has repeatedly published the same research in many different venues. Little or no additional information is presented in each of the multiple publications or presentations. Accordingly AMEC has determined that these citations are not critical to RfD derivation. Of the three other TOXLINE® citations, one concerned the mutagenicity of m-phenylenediamine and one was a review of the toxicology of hair dyes (in Italian), and the third was an abstract of a case study for resorcin dermatitis presented at a dermatological meeting. None of these three were considered relevant for RfD derivation.

A summary of the reports ultimately obtained and reviewed to support a resorcinol RfD is given in Table 2 (Human Studies) and Table 3 (Studies in Whole Animal and Other Cellular Systems). A majority of these studies are discussed in the text of this report.

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 natural phenolic compound that has also been synthesized, 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 (IARC 1977, 1999).

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-m³/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.

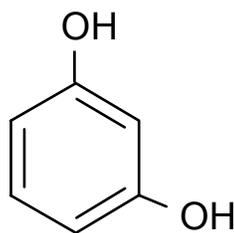


Figure 1. Structure of Resorcinol

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 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:

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

Resorcinol is an active ingredient in a number of hair dyes. Resorcinol is 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 humans, in the qualitative or quantitative handling of the compound. Thus, pharmacodynamics notwithstanding, 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 much lesser extent through intact skin. Absorption through damaged skin, as was the case for the subjects of many of the case studies in the literature, has not been measured but is presumably much higher.

Garton and Williams (1949) reported a study of the disposition of orally administered resorcinol in rabbits. In this study, approximately 76 per cent 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 [¹⁴C]-resorcinol after oral administration to male and female rats. Greater than 90 per cent 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 preparations (i.e., applied to the skin) containing high concentrations of resorcinol. In most of these cases, high concentrations of resorcinol had been applied as an ointment or solution to ulcerated or otherwise damaged skin (Hart and MacLagan,

1951; Hobson, 1951; Marsh, 1951; Thomas and Gisburn, 1961; Lundell and Normand, 1973) and might therefore be expected to be 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 per cent resorcinol in a hydroalcoholic vehicle 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 per cent of the applied dose (average, 1.64 per cent). This suggests resorcinol was absorbed into the system through the intact skin.

Yeung et al. (1983) also conducted *in vitro* skin penetration studies. The studies utilized excised human skin in a permeation apparatus. *In vitro* skin penetration occurred at a rate of 0.86 µg/cm²/hour. The authors state that this rate was in good agreement with the *in vivo* penetration rate of 0.37 µg/cm²/hour, calculated using the dose recovered from urinary excretion experiment described previously. These results indicate that resorcinol penetrates intact skin, although this route of exposure is less efficient than the oral route.

Wolfram and Maibach (1985) studied the dermal penetration of resorcinol in a hair dye matrix (Miss Clairol Black Velvet) through human and monkey scalp skin. An unspecified amount of radio-labeled resorcinol was added to the hair dye, which contained 1.2% resorcinol. The product's hair dyeing instructions were followed in dyeing the hair of three human volunteers and three Rhesus monkeys. Immediately after the hair dyeing, the hair was removed with an electric clipper so as to measure the skin penetration of resorcinol only during the hair-coloring procedure. After hair coloring, urine was collected from human volunteers and Rhesus monkeys. Radioactivity was determined by scintillation counting. Of the total dose applied, 0.076% and 0.177% of the resorcinol was excreted in the urine of humans and monkeys, respectively. These values were corrected for incomplete excretion based on previous work by the researchers. The half-life of excretion was 31 hours in both species. The flux of resorcinol

penetration through the human scalp was 2.2×10^{-10} mol/cm²/hour, which is equivalent to 0.024 µg/cm²/hour.

4.2. DISTRIBUTION

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

Oral exposure to carbon-14 labeled resorcinol ([¹⁴C]-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 Matthews, 1987). The majority of the dose was detected in excreta (90 per cent in urine, 2 per cent in feces) within 24 hours of dosing. These findings led Kim and Matthews (1987) to conclude that the compound would be rapidly cleared and not accumulate in any organ.

Merker et al. (1982) dosed rats with [¹⁴C]-resorcinol by the subcutaneous injection route, either as a single dose of 10, 50, or 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 that 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 in liver and kidney at the shorter time periods, particularly in pretreated animals¹. Low dose (10 mg/kg) distribution studies showed 62 per cent of the resorcinol equivalents (parent compound and/or metabolites) recovered in the urine at 1 hour, 7.1 per cent in the gastrointestinal tract, 2.3 per cent in the muscle, 0.72 per cent in the kidneys,

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

and 0.48 per cent in the liver. At the 50 and 100 mg/kg dose levels, only 0.2 per cent to 0.3 per cent 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, reporting a “fast” half-life ($t_{1/2}$) of 18-21 and 31-32 minutes for the single dose and multiple dose treatments, respectively, and slower rate with a $t_{1/2}$ of approximately 8.6 to 10.5 hours for the single dose treatment and 5.0 to 7.3 hours for the multiple dose treatment. No hypothesis for the biphasic nature of the elimination is provided. However, it should be remembered that the subcutaneous injection route of exposure generally results in slower release of compounds than oral or dermal exposures and may play a part in the biphasic kinetics.

4.3. METABOLISM

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

Kim and Matthews (1987) investigated the fate of [^{14}C]-resorcinol after oral administration to male and female rats. Greater than 90 per cent 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 conjugates². 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.

² Acid conjugation occurs at either or both hydroxyl moieties on the resorcinol molecule, leading to five possible permutations of glucuronic- (mono-, di-), sulfate- (mono-, di-), or mixed acids of resorcinol.

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 water-soluble conjugates. Studies in animals suggest that 76 per cent (Garton and Williams, 1949) to greater than 90 per cent (Merker et al., 1982; Kim and Matthews, 1987) 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 humans, 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.

5.1. HUMAN

Case reports, epidemiologic studies, and clinical testing of resorcinol in humans are described in this section. Much of these data exist because resorcinol has been used for therapeutic purposes for many years and is readily available to the public in a variety of cosmetic and over-the-counter personal care products. The Cosmetic Ingredient Review (CIR), an expert panel of independent scientists, periodically reports on the safety assessment of cosmetic ingredients.

In 1985 (CIR, 1985), this panel judged resorcinol to be safe for cosmetic use, i.e., dermal application of resorcinol in concentrations of roughly 0.1-1 per cent (1,000 -10,000 mg/L in aqueous or alcoholic solutions or 1,000 - 10,000 mg/kg in solid vehicles such as petrolatum). Thus, extensive experience with exposure to resorcinol exists.

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.

Poisoning reports in the clinical literature also indicate that acute high doses in children produce methemoglobinemia and hemolytic anemia (Cunningham, 1956; Deichmann and Keplinger, 1963; Lundell and Nordman, 1973; Tush and Kuhn, 1996). Such effects have been seen following topical treatments to damaged skin (e.g., eczema).

Due to the association between exposure to certain oxidants and pharmaceuticals and methemoglobinemia, Currie et al. (1997) assessed the potential of an external vaginal anti-itch cream that contained 20 per cent benzocaine and 3 per cent resorcinol to cause methemoglobinemia in women. The women were instructed to apply a 1-inch strip of cream, three to four times a day for seven days. Mean methemoglobin levels were similar before and after treatment. Currie et al. (1997) reported that the 1-inch strip of cream contained approximately 1.1 grams of benzocaine. Recognizing that the cream was 20 per cent benzocaine, the total amount of cream applied during each event was approximately 5.5 grams with 0.17 grams resorcinol. The total daily applied dose of resorcinol was estimated by AMEC as 11.3 mg/kg-day, assuming four treatments per day and a body weight of 60 kilograms.

Tush and Kuhn (1996) reported a case of methemoglobinemia in a six-day-old infant who had been treated for diaper rash with a vaginal anaesthetic cream containing 5 per cent benzocaine

and 2 per cent resorcinol. A “small amount” was applied five times over 1½ days. An estimate of the infant’s dose was made by AMEC based on the following assumptions. While Tush and Kuhn (1996) only reported that a “small amount” of cream was used, AMEC assumed that the amount would not be vastly different from the 1-inch strip of cream described in Currie et al. (1997). Thus, assuming a concentration of 0.11 grams resorcinol each event (2 per cent of 5.5 grams), 3.3 applications per day (5 times over 1½ days), and a body weight of 3.35 kilograms, the daily-applied dose of resorcinol was estimated as 108 mg/kg-day.

Autret et al. (1989) reported a case of methemoglobinemia in a two-year old child who had accidentally ingested 10 ml of Nestosy, a drug used for dental pain. Nestosy contains butoform, benzocaine, resorcin and 8-hydroxyquinoline. With the exception of butoform, all of the other components have been shown to be associated with methemoglobinemia so it is not known if resorcin was responsible for or contributed to the effect.

5.1.2 Dermal Toxicity and Sensitization

The use of high concentrations of resorcinol ointments (up to 50% resorcinol) for chemical “peels” is evidence of the ability of the compound to affect human skin at high concentrations. Resorcinol peels are used to treat hyperpigmentation, erythema and scars that occur from acne, as well as treatment for melasma, sun-damaged skin and freckles (Karam, 1993). A peel containing 50 per cent resorcinol may be applied for up to 30 minutes on the face and up to 1 hour on the back. The peel is spread over the skin and gently rubbed with a tongue depressor to increase absorption. Often two or more peels are applied after a few hours to two days.

The industrial hygiene literature contains reports of dermatoses in workers using resorcinol-based adhesives (Abbate et al., 1989). It is not possible to determine what proportion of the exposed population responds in this manner because the Abbate et al. (1989) study applied to a group self-selected for participation. Resorcinol-based adhesives may contain concentrations of resorcinol as high as several percent. At concentrations typical of mild therapeutic treatments, no irritation was seen in human volunteers (1.4 per cent, or 14,000 mg/kg, in a cosmetic product; CIR, 1985³).

³ The 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 appears to be a sensitizer, producing allergic dermatitis in a small proportion of individuals exposed repeatedly to the compound in cosmetic and pharmaceutical products (Eierman et al., 1982; Vilaplana et al., 1991; Nakagawa et al., 1992; Pecegueiro, 1992; Massone et al., 1993; Barbaud et al., 1995). Fisher (1982) characterizes this as a rare event, and Eierman et al. (1982) report that in a prospective study of dermatitis (487 cases in 179,800 dermatology patients) from any cosmetic, only 8 per cent of the cases were allergic reactions. Challenge of 149 of the dermatitis cases revealed only one positive reaction to resorcinol. Goossens et al. (1999) reported on data from 475 patients with allergic contact reaction to cosmetics. Only one case out of the 475 cases was allergic to resorcinol. Four out of 839 patients or 0.5 per cent had an allergic reaction to resorcinol when patch tested over a seven-year period (Tarvainen, 1995). Patch tests were also used to evaluate sensitization to allergens, vehicles and preservatives present in creams and ointments used to treat leg ulcers (Kokelj and Cantarutti, 1986). Among 25 patients tested, resorcinol, considered a vehicle, did not test positive. Guerra et al. (1992a) challenged individuals with contact dermatitis with resorcinol. Only 0.4 per cent (one case out of 261 clients) tested positive to resorcinol (Guerra et al., 1992a). In hairdressers with dermatitis, Guerra et al. (1992b) reported only a 1.3 per cent incidence of sensitization to resorcinol. Frosch et al. (1993) reported 0.6 per cent sensitization to resorcinol among European hairdressers. Resorcinol content of cosmetic products is typically 0.1 - 1 per cent (1,000 - 10,000 mg/kg) and may be greater (CIR, 1985). Allergic reactions at lower resorcinol concentrations of environmental relevance would presumably be more rare. Contact sensitization is not a systemic effect, because it occurs only in the skin at the point of contact. It does not occur after systemic doses. Therefore, dermal contact sensitivity is not a concern when deriving an oral RfD.

5.1.3 Thyrotoxicity

It has been reported that humans using topical resorcinol products containing high concentrations of resorcinol often on ulcerated or otherwise damaged skin for prolonged periods may experience interference with thyroid function, including goiter (Bull and Fraser, 1950; Hart and MacLagen, 1951; Hobson, 1951; Thomas and Gisburn, 1961; Katin et al., 1977). Lynch et al. (2002) have recently reviewed the thyrotoxic actions of resorcinol and report that thyrotoxicity in humans appears to derive from exposures that are both prolonged and continuous in terms of

the release of resorcinol. The dependence of the effect on the type of exposure has also been observed in laboratory animals (Arnott and Doniach, 1952; Samuel, 1955). Additional information on thyrotoxicity studies in animals is presented in Section 5.2 of this report. Evidence of hypothyroid effects (decreased iodine uptake, reduced circulating thyroxine levels, increased levels of thyroid stimulating hormone) and goiter (increased thyroid organ weight) resulted from acute and subchronic dosing of rodents with resorcinol but only when prolonged-release mechanisms of dosing (injection of oil-based solutions or resorcinol esters, topical application of ointments) were implemented (Samuel, 1955). It thus appears that, because resorcinol is so rapidly cleared, 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 cause thyrotoxicity (Samuel, 1955).

Bull and Fraser (1950) described three case studies where patients were treated with a resorcinol ointment for leg ulcers. In each case, patients exhibited signs of myxoedema or hypothyroidism. Additional examination of these patients confirmed enlarged thyroid glands (goiter). Whereas spontaneous myxoedema usually develops very slowly, the onset of the myxoedema symptoms in these three cases was very sudden, suggesting exposure to an antithyroid agent. Resorcinol ointment was considered the likely agent not only because all three patients were using the ointment, but also because myxoedema symptoms subsided and thyroid function returned when the ointment applications were withdrawn.

Resorcinol dosage was not reported in case one. In case two, the leg ulcers were treated daily over a two-year period with an ointment containing 12 percent resorcinol in glycerin, paraffin and lanolin. The daily treatments amounted to approximately four to six ounces per week. Assuming an application of six ounces per week of a 12 percent ointment and a body weight of 75 kilograms as reported by Bull and Fraser (1950) and assuming that the ointment had a density of 1.2 g/cc, AMEC has estimated the daily-applied dose as 47 mg/kg-day.

In case three, the varicose ulcer was treated for three years with a four percent resorcinol ointment (application amounted to approximately one pound per week). Assuming an application rate of one pound per week of a four percent ointment and a body weight of 70 kg, as reported by Bull and Fraser (1950), AMEC has estimated the daily-applied dose as 37 mg/kg-day.

Hart and Maclagan (1951) reported a case study of a woman who suffered from extensive ulceration of both legs. She self-treated the ulcers daily with a proprietary ointment when skin grafts and other medical treatment failed. Although symptoms of myxoedema were evident after seven years of her daily treatment, diagnosis was not made until after another five years. The link to resorcinol also was made at this time based on the findings reported by Bull and Fraser (1950) and from discovering that the proprietary ointment contained resorcinol. The myxoedema symptoms subsided and the thyroid goiter disappeared after treatment with resorcinol ointment was discontinued. The proprietary ointment contained lanolin (39.3 per cent), soft paraffin (46.7 per cent), zinc oxide (10.0 per cent), resorcinol (4.0 per cent) and cresol (0.002 per cent). Due to the lack of information on the application rate of the ointment, it was not possible to estimate a daily dose.

Hobson (1951) reported a case study of a man who suffered from leg ulcers associated with varicose veins. The ulcers were self-treated daily with a proprietary ointment, containing four percent resorcinol. After approximately nine months of applying this formulation, the ulcers were treated daily with an ointment containing 12 per cent resorcinol. Within two months, the patient's thyroid enlarged to four times normal size and the patient experienced myxoedema symptoms. Approximately six weeks after treatment with resorcinol ointment had ceased, the patient's goiter had noticeably shrunk and no symptoms of myxoedema were present. Due to the lack of information on the application rate of the ointment, it was not possible to estimate a daily dose.

Thomas and Gisburn (1961) reported a case study of a woman who self-treated her leg ulcers with ointment containing 12.5 percent resorcinol and 12.5 percent glycerin in soft paraffin over a 13-year period. As the ulcerations became more extensive, the amount of ointment used increased such that by the end of the 13-year period, up to a 1.5 pounds of ointment was applied every ten days. Myxoedema symptoms started six years prior and were initially relieved with thyroid tablets. When examined six years later, diagnosis was made of hypothyroidism again and of exogenous ochronosis, which is defined as a darkening of the cornea and of the skin of the face and hands. Thomas and Gisburn (1961) linked both diseases to the treatments with resorcinol ointment. While Thomas and Gisburn (1961) did not report the patient's weight, they reported the patient to be obese. Assuming a body weight of 100 kilograms, an application

rate of 1.5 pounds every ten days of 12.5 percent ointment, AMEC has estimated the daily-applied dose as 85 mg/kg-day.

Katin et al. (1977) observed hypothyroidism in a 70-year-old male patient on chronic hemodialysis and reported that the patient over a three-month period had applied skin cream containing two percent resorcinol to treat pruritus (itching), a frequent condition in hemodialysis patients. It was reported that the patient's skin was intact, but it was dry and contained multiple senile keratoses. Senile keratoses are premalignant warty lesions occurring on the sun-exposed skin of fair-skinned individuals. The patient had applied as many as three, 2.5-gram tubes per day. The patient's hypothyroid condition improved upon the discontinuance of the skin cream, suggesting that the resorcinol cream may have contributed to thyroid abnormalities. Assuming a body weight of 70 kilograms and an application rate of 7.5 grams per day of a two percent ointment, the daily-applied dose of resorcinol was estimated by AMEC as 2.1 mg/kg-day. Katin et al. (1977) noted that the patient was taking at least four medications upon admission (digitoxin, fludrocortisone acetate, multivitamins, and secobarbital).

The Lynch et al. (2002) review noted that there was no evidence of thyroid disturbance in occupational epidemiology studies of workers in resorcinol production plants and industries using resorcinol-based adhesives. No dose estimates could be derived from the occupational surveillance.

Roberts et al. (1990) investigated the occurrence of hypothyroidism among textile workers at a facility plant where resorcinol and thiourea were used in dyeing and finishing processes. Workers from all departments were asked to participate in the study. One hundred fifteen subjects were process workers and 122 were from other departments. Questionnaires were administered to all subjects to collect information on any history of thyroid disease, length of employment, etc. Blood samples were collected to measure thyroid stimulating hormone (TSH) levels as well as thyroid antibodies (antimicrosomal and antithyroglobulin antibodies). Among the 237 workers, only 15 were found to have any type of thyroid function abnormality. Of the 15, 3 cases were excluded due to preexisting thyroid conditions. Of the remaining 12, no cases of hypothyroidism were identified. Four had elevated TSH levels with minor non-specific symptoms, and the remaining 8 had elevated thyroid antibodies with all other measures of thyroid function normal (Roberts et al., 1990). According to the study authors: "...we were

unable to demonstrate a statistically significant occurrence of biochemically and immunologically detectable disturbances in thyroid function among the workforce....”

Seven of the twelve individuals with elevated TSH levels or elevated thyroid antibodies, were in the control group and designated as “unexposed,” because they were management, administration, and office staff. The study authors postulated that these individuals might have been historically exposed to thiourea and resorcinol because these individuals had worked in offices adjacent to ventilation outlets from finishing rooms. An attempt to measure these compounds in office air “some months” after the thyroid testing was done was negative. No exposure information from processing areas or office areas is available from the Roberts et al. (1990) study.

Tabershaw Occupational Medicine Associates (TOMA) conducted health studies of workers at three Koppers Company, Inc. plants in Petrolia, Pennsylvania (TOMA, 1978, 1980), in Raleigh, North Carolina (TOMA, 1981) and in Bridgeville, PA (TOMA, 1982). The Koppers Company, Inc. plant in Petrolia, Pennsylvania was a facility that manufactured resorcinol for more than 30 years. The chemicals of interest to which workers were exposed at this plant included benzene, by-products of benzene sulfonation, formaldehyde, and resorcinol. The Koppers Company, Inc. plant in Raleigh, North Carolina was a facility that produced laminated timber for construction purposes. The Raleigh plant was not a resorcinol production facility. It was a facility that used adhesive resins containing methanol, phenol, resorcinol, and paraformaldehyde. In addition, wood was treated at this facility from 1968 to 1975 using pentachlorophenol in liquefied petroleum carrier, and laminated timber used wood treated with chromium and arsenic wood preservatives as a starting material. This wood was treated off-site. The Koppers Company, Inc. plant in Bridgeville, Pennsylvania was a facility that primarily produced phthalic anhydride, polyester resins, and alkyd resins. Maleic anhydride and amino resins were also produced there. The Bridgeville plant was not a resorcinol production facility. It was a facility that used resorcinol as well as other chemicals in the production of resins for the chemical, paint and coatings, and plastics industries. The other chemicals used at the Bridgeville plant included acrylamide, dimethylaniline, dimethylformamide, benzene, hydroquinone, anhydrides, and acrylonitrile.

TOMA (1978) investigated the general health of the workers and the possible health effects from occupational exposure to resorcinol, benzene, by-products of benzene sulfonation, and formaldehyde, while TOMA (1980) was designed as a mortality study to determine whether mortality among the workers was significantly different from the general population. The overall health of the workers was evaluated with particular attention given to thyroid function. In addition to a physical examination of the thyroid, TSH and thyroxine were measured in 280 workers. Hormone levels in only seven workers were slightly out of the normal laboratory range, and two workers had a palpable thyroid. Although no statistical significance testing was performed, according to TOMA (1978), up to five percent of the general population may have a palpable thyroid, but only one percent of the workers had a palpable thyroid. Thus, the palpable thyroids observed were reported by TOMA as being not out of the ordinary. A nodular or goiterous thyroid was considered by TOMA to be abnormal, but no nodular or goiterous thyroids were observed among the workers nor were any signs or symptoms of hypo- or hyperthyroidism.

The normal laboratory TSH range for the participating laboratory was stated by TOMA as 2.0 – 10.0 μ units/ml. TOMA reported that levels of TSH in 274 workers were within normal range. No data were presented in the report for these workers. Only their TSH status as 2.0 – 10.0 μ units/ml was noted. Of the seven workers having hormones out of the normal range, TSH levels in six workers were 13.0, 11.0, 11.0, 11.0, 1.8 and 1.4. Thyroxine levels were normal for these six workers. In the seventh worker, the thyroxine level was reported at 3.9 μ g/100 ml. According to TOMA, the normal range for the participating laboratory was 4.5 – 13.5 μ g/100 ml. The TSH level was normal in this worker. TOMA also stated that in cases of hypothyroidism, thyroxine levels are 0 – 2 μ g/100 ml and TSH levels are greater than 40 μ units/ml.

AMEC notes that the normal ranges of thyroid hormones published in 2004 by the National Library of Medicine (2004) differ from those reported by TOMA in 1978. The normal laboratory TSH range for the participating laboratory was stated by TOMA to be 2.0 – 10.0 μ units/ml. The National Library of Medicine states that in 2004 the normal range is 0.4 to 4.0 μ units/ml for those with no symptoms of an under- or over-active thyroid. Similarly, according to TOMA, the normal range for the participating laboratory for thyroxine was 4.5 – 13.5 μ g/100 ml. The National Library of Medicine states that in 2004 the normal range for thyroxine is 4.5 – 11.2 μ g/100 ml. While there is not much disparity between the 1978 laboratory normal range for

thyroxine and the 2004 normal value, there is a large difference between the TSH normal values reported in 1978 by the laboratory used by TOMA and the 2004 value reported by the National Library of Medicine. According to TOMA (1978): "The limits of these 'normal' ranges established by the laboratory depend on the techniques used, the clinical populations served by the laboratory and the distribution of their data." The TOMA (1978) report provides no information on the laboratory techniques used in 1978 to allow a comparison to techniques current today.

TOMA (1978) concluded: "Although a few measurements are out of the normal range, thyroid function among the Petrolia population was within normal distribution and did not reflect any resorcinol hazards from the work environment..." No exposure information was reported in TOMA (1978), so no NOAEL or LOAEL for any health effect could be defined from this study.

In the second study of the Petrolia plant, which manufactured resorcinol, a group of 763 males made up the cohort in TOMA's (1980) mortality study. The average length of employment for this cohort was approximately 10 years. In an attempt to determine if there was a correlation between exposures and mortality, job titles for each worker were classified into one of 16 areas of the plant. In addition to a mortality analysis of the entire cohort, mortality analyses were done for selected areas as well. Results of the study showed that the overall mortality for the cohort of 763 workers at the Petrolia plant was 22 per cent less than expected based on a comparison to United States national age-sex-year-cause-specific mortality rates for 5-year time periods from 1925 to 1975. Furthermore, none of the 200 specific Standardized Mortality Ratios (SMRs) were significantly higher than the norm, and total cancer incidence was 67 per cent of the expected value. One death from thyroid cancer was reported. The expected number was 0.05 based on national statistics. The SMR was not statistically significant (TOMA, 1980). Results of the mortality analyses for selected areas showed no excess mortality for any specific cause among workers who were exposed to resorcinol or resorcinol intermediates.

TOMA (1980) concluded: "In conclusion, there is no evidence of mortality excess in any specific causes examined among the male workers at the Koppers' Petrolia plant, either taken as a whole or classified into various work areas according to their occupational exposures." No resorcinol exposure information was reported in TOMA (1980), so no NOAEL or LOAEL for any health effect could be defined from this study.

The health evaluation conducted at the Raleigh, North Carolina plant (TOMA, 1981), at which workers used adhesive resins containing methanol, phenol, resorcinol, and paraformaldehyde, involved 104 workers. Similar to the worker evaluation at the Petrolia plant, TOMA (1981) investigated the overall health of the workers including consideration of thyroid function by measuring levels of TSH and thyroxine. All levels were within the normal range. In this report, TOMA (1981) reported the normal range of serum TSH as 1-10 IU/L and the normal range of thyroxine as 4.5-12.5 µg/dL. The TSH value appears to be in error. It should be reported as 1-10 *milli*IU/L, which is equivalent to 1-10 µunits/ml. In addition, no individual worker's data are presented. It is only reported that all workers hormone levels fell within these normal ranges. AMEC notes that in the Petrolia study (TOMA, 1981), the normal laboratory TSH range for the participating laboratory was stated by TOMA to be 2.0 – 10.0 µunits/ml, and the National Library of Medicine states that in 2004 that the normal range is 0.4 to 4.0 µunits/ml for those with no symptoms of an under- or over-active thyroid. Both of these “normal” ranges differ from the “normal” range reported in the TOMA (1981) study.

TOMA (1981) concluded: “Thyroid function was estimated by blood levels of T₄ [note added: T₄ is thyroxine] and TSH (thyroid stimulating hormone). No abnormal values were observed.” No resorcinol exposure information was reported in TOMA (1980), so no NOAEL or LOAEL for any health effect could be defined from this study.

In the health evaluation at the Bridgeville, Pennsylvania plant (TOMA, 1982), which was a facility that used resorcinol as well as other chemicals, such as acrylamide, dimethylaniline, dimethylformamide, benzene, hydroquinone, anhydrides, and acrylonitrile, in the production of resins for the chemical, paint and coatings, and plastics industries, the thyroid function of 88 workers was investigated. No abnormal TSH levels were reported. In this report, TOMA (1982) reported the normal range of serum TSH as <10 mIU. This is taken by AMEC to mean <10 mIU/L. This is similar to the “normal” range reported by TOMA (1981). According to TOMA (1982), thyroxine levels were “slightly” elevated in two workers and “slightly” depressed in one worker (TOMA, 1982). In this report, TOMA (1982) reported the normal range of thyroxine as 4.7-12.1 µg/dL. This is similar to the “normal” range reported by TOMA (1978) and TOMA (1981). No individual data were provided, only the workers' status as “normal” or “abnormal.” TOMA (1982) does note that the two “abnormal” thyroxine values were seen in administrative

workers and one was seen in a shipping worker. According to TOMA (1982), these levels were “readily explained by history and examination.” However, no information was provided to explain this statement.

TOMA (1982) concluded: “There were no diseases or abnormalities found that can be definitely related to effects of work exposure...There was no evidence of thyroid or immunological abnormalities...There were several workers who had abnormalities, generally minor, not related to work. Each worker was counseled about the findings and advised to see his/her personal physician as appropriate.” No resorcinol exposure information was reported in TOMA (1982), so no NOAEL or LOAEL for any health effect could be defined from this study.

West and Stafford (1997) performed a case-control study of a small number of ordnance factory workers who had been diagnosed with certain blood abnormalities and exposed to resorcinol and other chemicals used in explosives manufacture. The blood abnormalities included neutropenia (low numbers of neutrophils), low platelet count, and macrocytosis (high numbers of macrocytes). Odds ratios were calculated for exposure to a variety of chemicals, including resorcinol. Four of the cases (14%) were exposed to low levels of resorcinol and fifteen of the controls (5%) were exposed to low levels of resorcinol. The odds ratio for low level exposure to resorcinol ranged from 0.9 to 9.2, which is not statistically significant. Zero of the cases (0%) were exposed to high levels of resorcinol and zero of the controls (0%) were exposed to high levels of resorcinol. No odds ratio could be calculated for this comparison. Exposure doses were not reported. Thus, no toxicological information on resorcinol is available from this study.

One research group has suggested high prevalence of goiter may be discernable in areas with elevated concentrations of resorcinol and similar compounds in water supplies (Gaitan, 1986,1988; Gaitan et al., 1987,1988). However, a specific exposure estimate from these reports is not possible. Further, Lynch et al. (2002) concluded that a causal association of goiter with resorcinol exposure cannot be established due to the presence of many other compounds in drinking water (including compounds that cause similar effects as resorcinol on thyroid hormone synthesizing enzymes), bacterial contamination of the water supplies, and confounding factors including nutritional status of the cohorts.

Lynch et al. (2002) noted that no thyrotoxic effect has been reported in the case study literature (dermal exposures) at doses lower than approximately 30 mg/kg-day for continuous exposures to compromised skin for long periods of time. They thus suggested that an applied dose of 10 mg/kg-day is a no-effect level for thyrotoxicity. Lynch et al. (2002) further state that systemic dose rates may have approached applied dose rates in the case study literature because of the compromised nature of the skin.

In a controlled study of dermal exposure, Yueng et al. (1983) applied a 2 per cent (20,000 milligrams per liter, mg/L) alcoholic solution of resorcinol to the skin of three volunteers (males over 18 years of age). The application was to approximately 2,600 square centimeters of skin (face, shoulders, upper chest and back) and was conducted twice a day, six days per week for four weeks. Measurements of thyroid hormone levels in blood collected from the volunteers at the end of weeks 1, 2, 3, and 4 of dosing were all within normal limits, not different from levels in samples collected from a concurrent control, and unchanged in time throughout the experiment. Yeung et al. (1983) estimated the dermal dose in this experiment to be approximately 12 mg/kg-day, which is similar to the no-effect level suggested by Lynch et al. (2002).

5.1.4 Summary of Human Data

In case studies of humans exposed to continuous, high levels of resorcinol for long periods of time, mostly to damaged skin, resorcinol has been reported to have caused central nervous system effects, methemoglobinemia, contact dermatitis, and thyrotoxicity. The case study literature, which involves individual cases and poor or no dose information, is not suitable for RfD derivation, but it does provide information about adverse effects that have been reported in humans. Several workplace health surveys among workers exposed to high levels of resorcinol for long periods of time have not reported adverse effects due to resorcinol exposure.

5.2. ANIMAL

The following sections summarize pertinent resorcinol studies in laboratory animals.

5.2.1 Acute and Subacute Toxicity

The following sections discuss toxicology studies that involve single dose experiments and experiments that range from a few days of exposure to several weeks exposure.

5.2.1.1 Lethality

Flickinger (1976) reported a series of acute studies in animals. The median acute lethal dose (LD₅₀) for resorcinol by single oral administration to rats is 980 mg/kg (95 per cent confidence intervals were 740 to 1,290 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 it was reported as mass of resorcinol per kilogram body weight, so it is not possible to determine the relative concentration of resorcinol that was applied to the skin. The dermal LD₅₀ derived from this experiment was 3,360 mg/kg (95 per cent confidence intervals 1,980 to 5,710 mg/kg). Flickinger (1976) also reported eye irritation using pure resorcinol in a rabbit eye test but no pulmonary irritation (measured by clinical observation) in rats exposed to as high as 2,800 milligrams of resorcinol per cubic meter (mg/m³) as an aqueous aerosol for eight hours. Flickinger (1976) also exposed rats, rabbits, and guinea pigs to resorcinol at 34 mg/m³ by inhalation six hours daily for two weeks, and no toxic effects were observed. Lloyd et al. (1977) reported no skin or eye irritation in rabbits exposed to resorcinol, and an oral LD₅₀ of 370 mg/kg in rabbits.

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 of 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 water 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, and thymus were weighed (data are reported as absolute weight and organ-to-body weight ratio), and those organs 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 hyperexcitability were observed transiently in male rats dosed at or above 225 mg/kg, while hyperexcitability was observed in female rats transiently at or above 55 mg/kg. Tachypnea (panting) was observed transiently in male rats at or above 225 mg/kg and in females administered doses of 110 mg/kg and 450 mg/kg. Hyperexcitability and tachypnea occurred within one-half hour of dosing and lasted for one to two hours. Tremors were transiently observed in mice (males at or above 150 mg/kg; females at 300 and 600 mg/kg). The tremors occurred within one hour of dosing and lasted one to two hours. Similar clinical signs have been reported in rats shortly after subcutaneous dosing in a range finding study preceding pharmacokinetic analysis (Merker et al., 1982). These investigators observed the effects in animals given 140 mg/kg but not those administered 88 mg/kg. Because the clinical signs subsided within about an hour of dosing, NTP considered the toxic signs to be due to acute central nervous system (CNS) effects of resorcinol that subside as the resorcinol is quickly cleared from the animals.

Body weight and body weight gain were similar to controls in all treatment groups of both species in the 17-day NTP study. The only statistically significant change in organ weights occurred in the high dose female rat group (decreased thymus weight and 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 short-term studies appears in a document prepared by the Cosmetic Ingredient Review (CIR, 1985). Rats were fed a diet containing resorcinol at a dose estimated at 261 mg/kg-day for four weeks (FEMA, 1979) or given two doses of 250 mg/kg by gavage, 24 hours apart (Hossack and Richardson, 1977). Clinical signs of the type described above were not reported in this study, but animals exposed by the dietary route had decreased adrenal-to-body weight ratios (this tissue was not investigated in the 17-day study by NTP).

Gatgounis and Walton (1962) investigated the sites of action of resorcinol within the CNS. A series of experiments in rabbits was conducted to compare responses to intravertebral and intracarotid arterial injections of resorcinol with the basilar artery ligated. The ligation separated cerebral circulation, making it possible to determine what region of the spinal cord was most sensitive to injections. When resorcinol (25 mg/kg) was injected into the vertebral artery (into low medullary areas), blood pressure increased in 28 out of 32 observations; when resorcinol (50 mg/kg) was injected into the carotid artery (into higher brain centers), no blood pressure response was detected in 22 of 27 observations. These results indicated that the low medullary spinal cord area of the rabbits' CNS was most sensitive to resorcinol. In another series of experiments, dogs received resorcinol injections into the vertebral, carotid and aortic arteries. Increases in contractile force and blood pressure were observed in all arteries. However, the dose, onset and intensity of response varied. Injections (10 mg/kg up to a total of 100 mg/kg) into the vertebral artery produced the most sensitive response. Injections into the carotid and aortic arteries were generally larger doses (50 mg/kg up to a total of 400 mg/kg) than injections into vertebral artery and the blood pressure responses were less. According to Gatgounis and Walton (1962), results from these series of experiments show that "resorcinol and isomers can act in the spinal cord to produce marked sympathetic circulatory effects."

5.2.1.3 Thyrotoxicity

Doniach and Fraser (1950) intraperitoneally injected rats with resorcinol at 1 to 10 mg/kg in one or two injections and reported a reduction in iodine uptake several hours later to 1/5 – 1/9 of the iodine uptake in controls. Resorcinol was also administered in drinking water as a 2 per cent solution, but no details were provided on: (1) the length of dosing, (2) the amount of drinking water consumed, (3) or the effects on iodine uptake.

Doniach and Logothetopoulos (1953) injected rats subcutaneously with pure resorcinol diacetate liquid and an aqueous solution of resorcinol diacetate at 0.4 and 1.0 mmol/100 gm in single doses. No effects were seen with the pure resorcinol diacetate. With the aqueous solutions, iodine uptake was reduced to 15.2 per cent and 19.8 per cent of the controls, respectively. The injection of 0.4 mmol maintained antithyroid action for approximately nine hours and the 1.0 mmol dose maintained action for 19 hours. To investigate the persistence of antithyroid activity when resorcinol was administered as an aqueous solution, the investigators

injected rats with 0.05 mmol/100 gm (55 mg/kg) of resorcinol in water and then injected labeled iodine three hours later. There was no statistical difference in iodine uptake between treated animals and controls. The authors concluded that the dose of resorcinol given as an aqueous solution had no appreciable antithyroid potency three to five hours after dosing (Doniach and Logothetopoulos, 1953).

Berthezène et al. (1979) fed rats (number, strain, weight and sex unspecified) a diet containing 5 per cent resorcinol by weight for 14 days. Upon sacrifice, it was found that among other effects, thyroid weights in the treated rats were greater than concurrent controls, plasma thyroxine levels were lower, and the half-life of thyroxine was reduced. Because only one dose was used in this experiment, it is not possible to establish a dose-response relationship. Further, due to the absence of information on the rats, dose estimation is difficult. However, based on standard consumption patterns in rats (i.e., an assumed rat body weight of 235 g and food intake of 21 g/day (TERA, 2002) the dose in these rats was likely to have been on the order of 4,468 mg/kg-day. Berthezène et al. (1979) suggested that resorcinol may affect (1) iodine uptake by the thyroid, (2) iodination of thyroglobulin (the precursor to thyroid hormones), (3) condensation of iodinated tyrosine residues of thyroglobulin (forming thyroxine), and (4) hormone release. Samuel (1955) discussed similar hypotheses, and *in vivo* studies by others support the effect of resorcinol on thyroglobulin iodination.

The oxidation of iodide and subsequent iodination of thyroglobulin is catalyzed by a peroxidase enzyme (thyroid peroxidase, TPO; Taurog, 2000) and it has been demonstrated *in vitro* in isolated porcine TPO that resorcinol inhibits this enzyme (Divi and Doerge, 1994; Doerge and Divi, 1995). Similarly, using pig thyroid slices and TPO extracted from pig thyroids, Lindsay et al. (1992) have demonstrated that resorcinol inhibits TPO and iodo-organification of thyroglobulin. Thus, the thyrotoxic action of resorcinol may be mediated by interruption of thyroid hormone synthesis.

5.2.2 Subchronic Toxicity

This section discusses toxicology information of studies that exceed several weeks in duration up to several months in duration. A section on general toxicology is followed by a section on thyrotoxicity.

5.2.2.1 General Toxicology

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 bolus gavage. In mice (B6C3F₁ 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 volume of 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 volume of 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. Body weights were measured weekly, at the termination of the study (final body weight) and at the time of necropsy. 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. Histologic pathology (microscopic examination) of all organs was conducted on the two highest dose groups.

A summary of significant findings is provided in Table 4. 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. However, the 520 mg/kg-day dose rate was not analyzed for either sex because there were few or no survivors.

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 decreased to a statistically significant level in male mice (both absolute weight and organ-to-body weight ratio), a statistically significant increase 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 (FEMA, 1979) reviewed by CIR (1985).

Kidney-to-body weight ratios (not absolute organ weights) were statistically elevated in female mice at the highest dose tested and in male mice at 28 mg/kg-day, but none of the higher doses. Brain-to-body weight ratios (not absolute weights) were elevated to a statistically significant extent in male mice only at doses of 112 mg/kg-day and higher.

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 animal 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 coincided with the rapid clearance of the compound and indicated to the NTP that the effect was 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).

In a recent study performed by WIL Research Laboratories and sponsored by the Resorcinol Task Force (RTF), male and female CrI:CD(SD)IGSBR rats were exposed to resorcinol in drinking water (WIL, 2003). The objective of this study was to identify doses of resorcinol that would cause minimal toxicity and yet allow for successful mating, gestation, parturition and lactation. This dose range finding study was a precursor to a guideline compliant two-generation reproductive study designed to adhere to the OPPTS-870.3800 and OECD 416 guidelines. As part of this preliminary study, the investigators examined the potential of resorcinol to induce neurotoxicity in F₁ offspring, evaluated the reproductive performance among the F₀ population and carried out a number of clinical and histological examinations, including examination of the thyroid. Groups of 14 rats per sex were administered resorcinol on a continuous basis at concentrations of 10, 40, 120 or 360 mg/L. Dosing occurred for 28 days prior to mating for both males and females. Dosing continued during mating, gestation, and lactation for all females, with exposure lasting for an average of 75 days. Males that were selected to be part of the interim study were exposed for 48 days, while the remaining males

were exposed for a total of 87 days. Offspring (F_1 litters) may have been exposed to resorcinol *in utero* or from nursing during post-natal days (PND) 0-21. One F_1 pup/sex/litter was administered resorcinol in drinking water from PND 21 to PND 28. Three F_1 pups/sex/litter were selected for behavioral testing and were not directly exposed to resorcinol.

The three F_1 pups per sex per litter selected for behavioral testing were evaluated for developmental landmarks and were subjected to several behavioral tests, including functional observational battery evaluations (monitored on PND 21), locomotor activity (monitored on PND 21 and PND 61), acoustic startle response (monitored on PND 20 and PND 60) and Biel maze swimming trials (monitored on PND 22 and PND 62) (WIL, 2003). Clinical analyses for thyroid-stimulating hormone (TSH), thyroxine (T_4) and triiodothyronine (T_3) were carried out on all F_0 rats at time of necropsy, on PND 28 for F_1 pups that were exposed to resorcinol, and on PND 4 for culled F_1 pups (WIL, 2003). Microscopic examination of F_0 thyroids was performed and brain measurements were performed on F_1 pups that were exposed to resorcinol or selected for behavioral testing. Results on the thyroid are discussed in the following section of this report.

No increase in mortality was reported. Transient increases and decreases in body weight were observed. WIL (2003) concluded that the body weight changes were not related to resorcinol because the changes occurred sporadically and the overall mean body weights were not affected. Exposure to resorcinol did not affect food or water consumption (WIL, 2003). The authors reported no impact on reproductive performance. Fertility indices were 100 per cent, 100 per cent, 85.7 per cent, 92.9 per cent and 100 per cent for controls, 10, 40, 120 and 360 mg/L groups, respectively (WIL, 2003). No changes in mean organ weights were reported. In the F_1 pups selected for behavioral testing, a decrease in brain width was reported in the 360 mg/L group F_1 females. No effects in brain length or weight were observed in these females and none of the three measurements were affected in the 360 mg/L male F_1 pups (WIL, 2003). The authors concluded that the decrease in brain width was not related to the resorcinol (WIL, 2003). No microscopic changes were observed in the brain in the F_1 pups selected both for exposure and behavioral testing (WIL, 2003).

Among the various sensory and behavioral tests conducted, statistically significant effects were observed only for the locomotor activity test. F_1 pups did not score differently from control animals for the functional observational battery evaluations, acoustic startle response and Biel

maze swimming trials (WIL, 2003). Locomotor activity increased in a dose-responsive fashion in males at PND 61. On the other hand, activity was not significantly increased in females at PND 61 or at PND 21 in either males or females. The authors concluded, "In the context of a dose range-finding study of limited power, the numerical increases in motor activity were not considered as conclusive evidence of a change in CNS status." (WIL, 2003).

AMEC investigated the biological significance of the locomotor activity test in light of the fact that this endpoint was the only neurobehavioral endpoint that was reported as significantly different from controls. AMEC's (2004) evaluation of the locomotor endpoint is appended to this report (Attachment A). AMEC concluded that while motor activity tests are reliable screening tools, there is a general consensus among the toxicology community that a single measure of activity is not sufficient evidence for neurotoxicity. In addition, AMEC conducted further statistical analyses on the locomotor data and concluded that there was no clear dose-response relationship. Because there was no clear dose-dependence, because statistical significance was caused by one or two outlier animals, because locomotor activity was the only neurobehavioral endpoint that was recorded as significantly different from controls, and because no effects on locomotor activity or other endpoints were seen in female animals or male animals of a younger age, AMEC agrees with WIL's conclusion that resorcinol in this study did not cause neurotoxic effects.

5.2.2.2 Thyrotoxicity

Several subchronic experiments have been conducted in rats and mice by the oral and subcutaneous dosing route to specifically evaluate the thyrotoxic properties of resorcinol.

In a recent study performed by WIL Research Laboratories and sponsored by the Resorcinol Task Force (RTF), male and female CrI:CD(SD)IGSBR rats were exposed to resorcinol in drinking water (WIL, 2003). The objective of this study was to identify doses of resorcinol that would cause minimal toxicity and yet allow for successful mating, gestation, parturition and lactation. This dose range finding study was in preparation for a guideline compliant two-generation reproductive study with full thyroid histopathology. The thyroid was a major focus of the study.

At interim necropsy, 38.7 per cent, 30.1 per cent and 42.4 per cent increases in mean TSH levels were reported for males in the 40, 120 and 360 mg/L groups, respectively (WIL, 2003). [Estimation of exposure dose in mg/kg/day is presented in Section 6.1.] These increases were not statistically significant and the TSH increases were not sustained in the males at the scheduled necropsy (WIL, 2003). T_3 and thyroxine levels in males were not different from control animals. In F_0 females, T_3 levels were increased in the 360 mg/L group, but thyroxine and TSH levels were not affected (WIL, 2003). The WIL authors indicated that the change in T_3 level "is inconsistent with the known mode of action of resorcinol (inhibition of peroxidase enzymes resulting in lower T_3 /thyroxine levels)." (WIL, 2003).

WIL (2003) reported minimal microscopic changes of the thyroid in the F_0 animals. The minimal changes were characterized as follicular cell hyperplasia. The incidence in the 360 mg/L group for males at interim necropsy was 6 animals out of 7 and for control 3 out of 7. At scheduled necropsy, incidence in males in the 360 mg/L group was reported as 5 out of 7; controls as 3 out of 7. In females in the 360 mg/L group, incidence was reported as 6 out of 14 and controls as 3 out of 13. The authors reported that none of these differences in incidence of follicular hyperplasia were statistically significant (WIL, 2003).

AMEC observed that the greatest frequency of thyroid follicular hyperplasia incidence was reported for males and females at the 360 mg/L dose level. Consequently, alternative statistical tests were evaluated to determine whether or not the results of any dose groups were statistically significantly different from the control group and whether a trend in hyperplasia incidence with increasing dose might be present. Odds Ratios were determined to be appropriate for this data set because these ratios are a simple way to compare whether the probability of a certain event is the same for two groups. An Odds Ratio of 1 means that there are no differences between the groups being compared. The further away from 1 the Odds Ratio is, the stronger the potential effect. Within each dose group, AMEC compared F_0 interim necropsy males to controls, F_0 scheduled necropsy males to controls and F_0 females to controls. For each comparison, the confidence limits for the Odds Ratios overlapped 1.0 indicating that there were no significant differences between test groups and the control group. Because of the low statistical power of the individual comparisons, it was decided to group animals to increase the statistical power of the tests. When both male groups were grouped together (i.e., interim and scheduled F_0 males), confidence limits of the Odds Ratios for this

larger group still overlapped 1.0 for all dose groups. When all animals were grouped together (i.e., interim and scheduled F₀ males and F₀ females), confidence limits of the Odds Ratios for this larger group still overlapped 1.0, except for the comparison of controls and the 360 mg/L dose group. This means that the high dose group could be designated a LOAEL for thyroid follicular hyperplasia if animal groups were combined for statistical significance testing purposes. In addition, AMEC applied the Jonckheere-Terpstra test, which is a commonly used non-parametric test to evaluate trends in data. The results also indicated a potential trend over all dose groups when the animals were combined into a single group for statistical significance testing purposes. AMEC's (2004) report is appended to this report (Attachment A).

The subchronic drinking water study in rats performed by the WIL Laboratories for the Resorcinol Task Force (RTF) was selected as the key study for derivation of an RfD because it is a fully documented study that measured multiple endpoints, including thyroid weight, circulating T₃, thyroxine, and TSH hormone levels, and thyroid histopathology. Thyroid effects, i.e., follicular cell hyperplasia, was selected as the critical endpoint for the derivation of an RfD for resorcinol. The thyroid hyperplasia was not accompanied by thyroid hypertrophy or changes in circulating thyroid hormones. The prevailing model for the mode of action for thyrotoxic chemicals is that alterations in circulating thyroid hormones (decreases in thyroxine and T₃, increases in TSH) are early biological effects that precede altered thyroid structure, which includes thyroid cell hypertrophy followed by hyperplasia (Capen, 1997, 1998, 2000; Hard, 1998). No adverse effects on thyroid function were demonstrated. The observed hyperplasia could have been artifactual, given the fact that no differences were observed in circulating thyroid hormones compared to controls.

In the NTP (1992) 91-day studies, male and female rats and mice (10 animals per group) were given one of five doses of resorcinol by gavage. In mice (B6C3F₁ 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 volume of 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 volume of 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. No changes in thyroid weight or relative thyroid weight were 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.

Although no thyroid effects were seen in any animal group, the NTP subchronic study was not as useful as the WIL (2003) data for RfD derivation for three reasons: (1) dosing was gavage bolus and not continuous, which would be more relevant to human risk assessment, (2) bolus dosing causes a peak and then rapid drop in the concentration of resorcinol in the thyroid tissue but thyrotoxicity appears to require a continuous dose of resorcinol in the thyroid tissue, (3) circulating thyroid hormone levels were only measured for male and female rats in the 13-week study and (4) only T₃ and thyroxine were measured, not TSH.

Seffner et al. (1995) reported changes in the quantitative morphology of the thyroid gland of BD x WELS/Fohm rats (n=12) provided low concentrations (40 mg/L) of resorcinol in drinking water for 84 days. The mean height of thyroid follicular cells was increased and the diameter decreased in treated rats *versus* concurrent controls (statistically significant by Student's t-test, p<0.05). No measurement of overt pathology or thyroid hormone levels were evaluated in this study and, because only a single dose level was employed, a dose-response relationship cannot be established. Seffner et al. (1995) provided no estimate of dose, but under standard assumptions, (i.e., rats consume approximately 15 per cent of their body weight in water per day, TERA, 2002), the study rats may have received on the order of 6 mg/kg-day. This study was not as useful as the WIL (2003) data for RfD derivation for several reasons: (1) no dose information was presented, (2) only a single drinking water concentration was tested, (3) no measurement of overt pathology or thyroid hormone levels was evaluated in this study, and (4) only changes in the size of thyroid cells were measured.

Cooksey et al. (1985) exposed rats (female Wistar strain, 95-105 grams, n=5) to resorcinol in drinking water. Cooksey et al. (1985) did not provide information on the concentration of resorcinol in water, but estimated the dose to be 9 micromoles per animal per day (approximately 10 mg/kg-day in a 100 g rat). Rats were given one microcurie of ¹²⁵Iodine after 30 days of resorcinol exposure and the animals were sacrificed 3 hours after radionuclide administration. The thyroid of each animal was weighed and measurements intended to evaluate thyroid hormone iodination were performed. Adjusted thyroid weights (mg per 100 g body weight) in treated animals appeared to be roughly 50 per cent greater than those of control

animals given resorcinol-free water (data provided only in graphic form). The difference was statistically significant by t-test ($p < 0.05$).

The ratio of ^{125}I iodinated thyroxine (^{125}I -thyroxine) and triiodothyronine (^{125}I - T_3) to total thyroxine and T_3 , respectively, was reduced (statistically significant; $p < 0.05$) in treated animals, while the ratio of free ^{125}I to ^{125}I -thyroxine and ^{125}I - T_3 was increased. Cooksey et al. (1985) interpreted these observations as evidence for reduced iodination of thyroid hormones in the presence of resorcinol exposure. The investigators provided no information on circulating levels of thyroid hormones or of thyrotropin, parameters that are more typically used for determining the presence of thyroid toxicity (Canaris et al., 2000). Because the uptake of iodine and iodination of thyroglobulin to produce thyroid hormones is quite efficient (Farewell and Braverman, 1996), the observations of Cooksey et al. (1985) represent an effect, but not necessarily one that connotes true thyrotoxic action. This study was not as useful as the WIL (2003) data for RfD derivation for several reasons: (1) no information was provided on the concentration of resorcinol in water, (2) only a single drinking water concentration was tested, (3) thyroid weights were presented only graphically, and (4) no measurement of overt pathology or thyroid hormone levels was evaluated in this study, only measurement of incorporation of radioiodine after a 3-hour bolus dose.

Gaitan (1986,1988) and Gaitan et al. (1987,1988) have suggested that the high prevalence of goiter in certain areas may be associated with watersheds and aquifers rich in coal and shale. Gaitan et al. (1993) studied the effects of a coal-water extract on rats. Buffalo rats ingested 20 mL/day of an aqueous extract of coal (50 mg/mL) for 8 weeks. On the last day, rats were injected with labeled iodine and then sacrificed. Body weights did not differ between treated and control animals. Thyroid weights were significantly greater in treated animals. Histological examination of control thyroids was normal; histological changes observed in the treated rats included smaller thyroid follicles, columnar epithelium and dense colloid. Iodine uptake was greater in treated animals. Phenol, resorcinol, phthalic acids, pyridines and PAHs are among compounds likely present in coal extracts. Gaitan et al. (1993) specifically selected the Buffalo rat because PAHs are known to cause thyroid effects in this strain of rat. The Gaitan et al. (1993) study is unsuitable for derivation of an RfD because it was not a study of resorcinol. Although resorcinol may have been present in the coal-water extract, many other compounds were also present, and the concentrations of resorcinol are unknown.

Doniach and Logothetopoulos (1953) cited one study where rabbits were injected subcutaneously with 50 mg/kg-day resorcinol in an aqueous vehicle for 15 days (Klein et al., 1950), and another study where mice were injected with the same dose every 48 hours for one month (Cheymol, et al., 1951). No thyroid hyperplasia was observed in either the rabbits or mice. In their study, Doniach and Logothetopoulos (1953) investigated thyroid effects in rats exposed to resorcinol by the dermal route of exposure. Thyroid weights were not different between controls and rats treated with a 12.5 per cent resorcinol ointment containing 12.5 percent glycerin, 37.5 per cent wool fat, and 37.5 per cent yellow soft paraffin. The ointment was applied to their shaved abdomens for fifteen minutes two times daily for three weeks. This study is not appropriate for RfD derivation because no dose information was provided, and dermal studies are generally not appropriate for deriving RfDs for use in evaluating human oral exposures.

In another series of experiments, Doniach and Logothetopoulos (1953) injected rats subcutaneously into their flanks with 0.14 mmol/100 gm body weight of 1.5 percent resorcinol in arachis oil twice daily. The animals were sacrificed at 10, 31, 47 and 69 days of treatment. One control animal sacrificed at day 47 had a thyroid weight of 16 mg, and thyroid weights of two controls sacrificed at day 69 were 18 and 13 mg. Thyroid weights of the 47-day and 69-day treated rats were 32 and 25 mg, respectively. The histology for the control thyroids was normal. Thyroids from treated animals exhibited hyperaemia, cellular hyperplasia and colloid depletion. This study is not appropriate for RfD derivation because the pharmacokinetics of resorcinol from subcutaneous injection experiments differs considerably from oral pharmacokinetics.

Doniach and Logothetopoulos (1953) conducted a final series of experiments where 0.4 mmol/100 gm of aqueous resorcinol acetate was injected subcutaneously into rats twice daily for up to 25 days. At 12 and 25 days of treatment, thyroid weights were 139 and 184.5 per cent of control weights, respectively. Upon examination, thyroids showed increased vascularity, cellular hyperplasia and colloid depletion. According to Doniach and Logothetopoulos (1953), the hyperplasia is due to an increased output of thyrotrophic hormone by the anterior pituitary as a result of a fall in thyroxine content in the blood. Antithyroid compounds stop thyroxine formation. Blood levels of thyroxine can be maintained for a few days as the body draws on stores of the hormone. When that source is exhausted, secretion of thyrotrophic hormone

continues. Doniach and Logothetopoulos (1953) further concluded, “an escape from even a few hours during the day from the antithyroid action of a drug, as occurred with the injection of resorcinol in water, owing to its rapid elimination, would be associated with temporary thyroxine formation. This thyroxine production would prevent or delay goitrogenesis in proportion to the amount of thyroxine synthesis and of available hormone already stored.” This study is not appropriate for RfD derivation because the pharmacokinetics of resorcinol from subcutaneous injection experiments differs considerably from oral pharmacokinetics.

5.2.3 Chronic

This section discusses toxicology information from studies that exceed 90 days in duration up to lifetime exposures. A section on general toxicology is followed by a section on thyrotoxicity.

5.2.3.1 General Toxicology

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 study 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 study, 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 study is provided in Table 5. Briefly, mortality, measured as mean survival days, was significantly affected at the highest dose in male and female rats, but not in 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. Similarly, the increased brain-to-body weight ratio observed in male mice in the 91-day study was not reproduced in the 2-year study, although this ratio was found to be elevated in male rats in the 112 mg/kg-day dose group. There were no statistically significant differences relative to control in the incidence of neoplastic or non-neoplastic lesions of the liver or brain in any treatment group evaluated.

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, as with the liver and brain, 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 transiently observed at the both administered doses in male and female mice (112 and 225 mg/kg-day) and in male rats (112 and 225 mg/kg-day) in the 2-year study. Female rats given 100 and 150 mg/kg-day in the 2-year study displayed the CNS effect, but it was not seen at 50 mg/kg-day. This observation has been reported in other animal 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 coincided with the rapid clearance of the compound and indicated to the NTP that the effect was an acute response.

No tumors were found to be present in dosed animals of either species or sex at incidences different from the control animals. These observations 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. As reported in CIR (1985), resorcinol has generally been negative in bacterial assays for mutagenicity (Ames et al., 1975; McCann et al., 1975; Anderson and Styles, 1978; Florin et al., 1980; Rapson, et al., 1980; Shahin, et al., 1980; Bracher et al., 1981; Crebelli et al., 1981; Gocke et al., 1981; Probt, et al., 1981), including those conducted by NTP preceding the chronic bioassay (NTP, 1992). *In vitro* tests in mammalian cells are mixed. Tests in certain mammalian cells, including hamster V79 cells and human lymphocytes indicate the compound does not produce sister chromatid exchange (SCE, an exchange of sections of the DNA strand between chromatid pairs often observed in genetic material treated with mutagens). Human lymphocytes treated *in vitro* with resorcinol were negative for SCE, but did show chromosomal aberrations (Darroudi and Natarajan, 1983; Ishihara et al., 1991). A mixture of p-phenylenediamine dihydrochloride, resorcinol and hydrogen peroxide was not mutagenic in the Ames test and the mouse lymphoma assay, and did not cause chromosome aberrations in human lymphocytes (Bracher et al., 1990). The same mixture without resorcinol was mutagenic in the Ames test and did produce chromosomal aberrations (Bracher et al., 1990). Additionally, NTP (1992) and McGregor (1988a,b) reported SCE in resorcinol-treated Chinese hamster ovary cells and trifluorothymidine resistance (an indicator of mutation) in mouse lymphoma cells.

The NTP (1992) reported that most *in vivo* assays of resorcinol chromosomal effects have been negative. For instance, *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). In a micronucleus test, the incidence of micronucleated erythrocytes was not increased in rats exposed to resorcinol at 500 mg/kg-day via the oral route (Hossack and Richardson, 1976).

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 given in the diet for 49 weeks (728 mg/kg-day) promoted the effect of the carcinogen methyl-n-nitrosamine given by intraperitoneal injection on tongue papillomas and esophageal squamous cell carcinomas in rats. Lung tumors were reduced, but not statistically significantly so. Hasegawa et al. (1990) studied the effects of resorcinol and several other antioxidants on induced lung and thyroid

tumors by N-bis(2-hydroxypropyl)nitrosamine (DHPN) in rats. Resorcinol did not reduce the number of lung tumors (other antioxidants did lower the incidence) and increased the incidence of thyroid adenomas. Resorcinol did not induce thyroid lesions by itself (Hasegawa et al., 1990). Rats received resorcinol at 0 or 0.2 per cent in diet after exposure to N-nitroso-butyl-N-(4-hydroxybutyl)amine, a bladder cancer initiator. Resorcinol alone did not induce bladder lesions nor did it enhance any tumor after the administration of the initiator (Miyata et al., 1985). Rats received 0 or 100 mg/kg resorcinol in their diet for six weeks after receiving N-nitrosodiethylamine, an initiator of liver carcinogenesis. No increase in enzyme-altered foci was observed (Stenius et al., 1989).

In another study, rats received resorcinol at concentrations of 0 or 0.8 per cent in their diet for 51 weeks (Hirose et al., 1989). A second group of rats was given the same concentration of resorcinol but after oral gavage of a known gastric carcinogen. Body weights were reduced in both groups. In the resorcinol-only group, mild forestomach hyperplasia was reported, but no forestomach tumors. Resorcinol did not increase the incidence of forestomach carcinomas in the initiated group. Shibata et al. (1990) investigated the effects of five phenolic antioxidants, including resorcinol, on the forestomach and stomach epithelium of rats. Resorcinol did not induce any changes, whereas the other phenolic compounds tested had cancer-promoting effects. Hirose et al. (1986) investigated the effects of 13 phenolic compounds, including resorcinol, on the forestomach epithelium of hamsters, which are more sensitive to such lesions. No activity of induction of forestomach hyperplasia or lesions from resorcinol exposure was observed. Maruyama et al. (1991) reported that resorcinol, as well as other phenolics tested, had an inhibitory effect on pancreatic carcinogenesis in hamsters. Resorcinol suppressed cyclooxygenase-2 promoter activity in colon cancer cells (Mutoh et al., 2000). Rats received resorcinol at 0 or 0.8 per cent in diet for 36 weeks either as resorcinol only or after rats were exposed to a known initiator of bladder cancer. Resorcinol alone did not induce bladder tumors nor did resorcinol increase the incidence of bladder lesions in rats initiated with the bladder carcinogen, N-butyl-N-(4-hydroxybutyl)nitrosamine (Kurata et al., 1990).

Dermal application of resorcinol in mice and rabbits with a solution containing up to 50 per cent 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) performed a carcinogenicity study of four resorcinol-containing hair care formulations (hair dyes) by the

dermal exposure route in Sprague-Dawley rats (see also, IRDC (1979)). The test materials were prepared by mixing equal volumes of the hair dye formulation and 6 per cent aqueous hydrogen peroxide. One-half milliliter of this test material containing 0.5 to 1 per cent resorcinol by weight was applied to the backs of male and female rats twice per week for two years. However, in addition to containing resorcinol at concentrations of 1-2 percent, the four hair dye formulations contained other active ingredients at total concentrations ranging from 15 to 23 per cent. These other ingredients included ammonia, phenylenediamines, diaminoanisoles, nitro phenylenediamines, and amino nitrophenols. No treatment-related tumors were identified for the resorcinol-containing preparations. However, the study is not discussed further, because the mixtures were very complex and there was no resorcinol-only test group.

5.2.3.2 Thyrotoxicity

The 2-year NTP (1992) study 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.

Thyroid weights were not recorded in the NTP chronic study in either rats or mice. In addition, thyroid hormones were not measured in either rats or mice. Thyroid histopathology was performed in rats and mice and no statistically significant differences between controls and treated animals were seen with regard to thyroid C-cell hyperplasia, follicular cell depletion, follicular cell hyperplasia or follicular cell metaplasia.

Although no thyroid effects were seen in any animal group, the NTP chronic study was not as useful as the WIL (2003) data for RfD derivation for three reasons: (1) dosing was gavage bolus and not continuous, which would be more relevant to human risk assessment, (2) circulating thyroid hormone levels were not measured, and (3) thyroid weights were not measured.

5.2.4 Reproductive and Teratology Studies

No oral multigeneration reproductive study of resorcinol was found in the published literature. However, the WIL (2003) study reported above, which was a comprehensive dose range finding study using the oral exposure route with drinking water as the experimental medium, was one phase of a comprehensive two-generation reproductive toxicity study currently in progress. Resorcinol at doses as high as 360 mg/L in drinking water did not affect F₀ reproductive performance as measured by estrous cycles, mating and fertility indices, mean number of days between pairing and coitus and mean length of gestation.

Burnett and Goldenthal (1988) evaluated four resorcinol-containing hair preparations applied dermally to Sprague-Dawley rats in a two-generation study. However, preparations, containing 0.5 to 1 per cent resorcinol also contained other compounds. These other ingredients included ammonia, phenylenediamines, diaminoanisoles, nitro phenylenediamines, and amino nitrophenols. There were no statistically significant differences between treated and control groups in any liter for any reproductive index. However, the study is not discussed further, because the mixtures were very complex and there was no resorcinol-only test group.

Resorcinol has been tested for teratogenicity and the studies have been largely negative. While Korhonen et al. (1983) saw weak effects of resorcinol on toxicity to chicken embryos, in more traditional teratogenicity assays by DiNardo et al. (1985), Kavlock (1990), and studies conducted by Hazelton Laboratories⁴, no teratogenic effects were observed in rats and rabbits given resorcinol by gavage.

In the Hazelton rat study (Hazelton, 1982a; Spengler et al., 1986), 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

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

(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 was not statistically significant. There was no effect of resorcinol on implantation. Early intrauterine loss was higher in all resorcinol-treated groups and was statistically significant by chi-square tests ($p < 0.05$). 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 per cent loss in controls versus 4.1, 7.2 and 3.8 per cent 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 by the authors 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 per cent, 7.7 per cent, 8.5 per cent, and 10.6 per cent 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 per cent) 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 were concordant with the results of DiNardo et al. (1985), who studied Sprague Dawley rats (10 to 13 per group) given 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 et al. (1985) did not present the raw data for the study, but indicated

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. It should be noted that while rat thyroid development starts around gestational day (GD) 9, fetal synthesis of thyroid hormone and development of neurobehavioral function occur after GD 15. Therefore, the rat teratology studies may have missed time periods for development that might be affected by resorcinol.

Kavlock (1990) studied a series of substituted phenols, including resorcinol, in order to develop a database for quantitative structure activity relationship (QSAR) modeling of developmental toxicity. Groups of 15 pregnant Sprague-Dawley rat dams each were given 333, 667, or 1000 mg/kg of resorcinol by oral intubation on day 11 of pregnancy. Maternal weight gain, litter size, perinatal loss, pup weight and litter biomass of the treated groups were compared to that observed in a group of 20 control dams and their litters. The only observed effect was a reduction of maternal weight in dams given 667 or 1000 mg/kg. No effect was seen in any measure of developmental toxicity. Application of the QSAR model derived from the overall findings suggested that developmental effects, defined as an increase in perinatal loss of one greater than controls, or a 10 per cent reduction relative to controls in litter weight by post-natal day 6, would require a maternal exposure in excess of 2000 or 1600 mg/kg, respectively.

In the Hazelton rabbit study (Hazelton, 1982b; Spengler et al., 1986), 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 killed and examined. Negative vehicle control and positive (Vitamin A 6 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. Pregnancy rates for controls and positive controls were 76 per cent and 83

per cent, respectively. Rates for treated animals were 65 per cent, 52 per cent and 68 per cent for the 25, 50, and 100 mg/kg groups, respectively. Two animals 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. Discounting these animals, post-implantation losses were found to be higher in the 25 and 50 mg/kg groups but not the high dose group. When the animals with total intrauterine deaths were included, post-implantation losses 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 total variations in control rats were high (80, 85.7, 88.2 and 84.3 per cent fetuses had skeletal variations in vehicle, 25, 50, and 100 mg/kg resorcinol groups, respectively), and total variations seen in treatment groups were not statistically significantly different from controls. Individual variations were also not statistically significantly different from controls. Two malformations were observed in the positive controls. 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. The sensitivity of the rabbit study may be questionable due to the pregnancy rates and to the low number of malformations among the positive controls.

5.2.5 Summary of Animal Data

Acute and subacute toxicity information is summarized in Section 5.2.1. Lethal dose studies demonstrate that resorcinol is not highly acutely toxic. In a 17-day gavage study in rats and mice, it was found that the resorcinol did not affect body weight in either species. Effects seen in various dose groups included mortality, transient CNS effects, and organ weight changes. Acute and subacute studies of thyrotoxicity in rats show that resorcinol injected intraperitoneally or subcutaneously or fed to them at levels exceeding 4,000 mg/kg/day can cause thyroid effects. Resorcinol has been shown in *in vitro* experiments to inhibit the porcine thyroid peroxidase enzyme. Similar results have been found in *in vitro* porcine thyroid tissue experiments.

Subchronic toxicity information is summarized in Section 5.2.2. In a 13-week gavage study in rats and mice, it was found that resorcinol did not affect body weight or the thyroid in either species. Effects seen in various dose groups included mortality, transient CNS effects, and organ weight changes. Several subchronic studies have addressed the issue of thyrotoxicity. It has been shown that resorcinol injected subcutaneously in oily vehicles causes thyrotoxicity in rats, but this effect is not seen when aqueous resorcinol is injected. Resorcinol administered in drinking water to rats was shown in two studies to cause thyrotoxicity, but only single doses were tested, no dose information was provided, and the relevance of the study endpoints was questionable. A recent subchronic study using the drinking water route of administration focused specifically on thyroid toxicity in addition to a comprehensive set of standard toxicity endpoints. The highest dose tested was found to be a NOAEL for body weight changes, organ weight changes, CNS effects, and all other effects, including changes in circulating thyroid hormones. Thyroid tissue hyperplasia was found in the highest dose group, and this dose can be considered either a NOAEL or a LOAEL for thyroid hyperplasia, depending on the manner in which animals are grouped for statistical analysis. Considering the high dose a LOAEL for this effect is conservative.

Chronic toxicity information is summarized in Section 5.2.3. In a 103-week gavage study in rats and mice, it was found that resorcinol did not affect body weight or the thyroid in either species. Effects seen in various dose groups included mortality, transient CNS effects, and organ weight changes. No tumors were found in animals of either species at levels different from control animals. Two skin carcinogenesis studies were performed in mice and rabbits, and resorcinol did not cause skin cancer in these animals.

Reproductive and teratology studies are summarized in Section 5.2.4. In one drinking water study in rats, resorcinol did not affect reproductive performance in the F₀ generation. In several teratology studies, resorcinol caused no developmental effects.

6. DOSE RESPONSE

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 Lowest Observed Adverse Effect Level. (LOAEL). Thus, an RfD is computed as follows:

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

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 (Renwick, 1993; IPCS, 1994; 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 EFFECT AND KEY STUDY

The critical effect for resorcinol has been defined as adverse effects on the thyroid. There are many reasons for defining thyrotoxicity as the critical effect. Thyrotoxicity has been observed in humans using resorcinol-containing pharmaceuticals at high doses for extended periods of time, and *in vitro* studies show that resorcinol can reversibly affect thyroid function. The impact of resorcinol on thyroid function has been discussed in a recent document issued by the European Commission entitled *Study on the Scientific Evaluation of 12 Substances in the Context of Endocrine Disrupter Priority List of Actions* (WRc-NSF, 2002). Specifically, WRc (2002) stated in the chapter on resorcinol:

In vitro studies indicate that the anti-thyroidal activity observed following resorcinol exposure is due to inhibition of thyroid peroxidase (TPO) enzymes, as evidenced by disruption of thyroid hormone synthesis and changes in the thyroid gland consistent with goitrogenesis.

Certain older *in vivo* laboratory animal studies have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high doses and required a vehicle (such as peanut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable.

Studies conducted as part of the National Toxicology Programme have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight⁻¹ day⁻¹ in rats and 450 mg kg body weight⁻¹ day⁻¹ in mice for 13 weeks and 150 – 225 mg kg body weight⁻¹ day⁻¹ for 5 days per week over 2 years in rats and mice.

There is evidence of effects on adrenal weights at all doses tested in NTP rat and mouse 13-week studies. However, the observed responses did not show dose-dependent relationships.

Currently available data indicate that resorcinol is not embryotoxic or teratogenic.

WRc (2002) also concluded: “The available exposure data indicate that resorcinol does not represent a risk to workers or consumers based on current exposure pathways.”

ATSDR (2004) issued a Public Health Assessment for the Bear Creek Chemical Area in which toxicology information on resorcinol was reviewed and evaluated. ATSDR (2004) reviewed the oral Reference Dose proposed by AMEC in 2003 (0.07 mg/kg-day) and found it to be “acceptable.” Accordingly, it did not evaluate oral toxicology information. The proposed Reference Dose reviewed by ATSDR pre-dates the availability of the recent study performed by

WIL Laboratories (2003) that specifically focused on the critical effect for resorcinol, which is thyrotoxicity.

Upon reviewing dermal and inhalation toxicology data, ATSDR (2004) concluded, “resorcinol is minimally toxic by the dermal and inhalation routes.” ATSDR (2004) acknowledged that adverse effects on the thyroid have been reported in some people who repeatedly used pharmaceutical preparations containing high levels of resorcinol for many years, but they concluded that such exposures are not equivalent to dermal exposure to resorcinol in water used for showering or bathing. In conclusion, ATSDR (2004) stated that dermal exposure to resorcinol in water is not likely to cause adverse effects on the thyroid.

Other recent literature reviews have also concluded that resorcinol's critical effect is thyroid toxicity. For instance, CANTOX (2000) in a study entitled *Resorcinol: Toxicological Review and Risk Assessment With Emphasis on Thyroidal Effects* stated:

The *in vitro* data demonstrate that resorcinol can inhibit thyroid peroxidase enzymes (TPO) and potentially block the synthesis of thyroid hormone. The results of older, non-Good Laboratory Practices (GLP) studies conducted in experimental animals show that resorcinol administered at high-doses by routes or methods (e.g., dermal, subcutaneous in oil vehicle, in drinking water, and in feed) that allow for continuous exposure (i.e., continued presence of resorcinol in the blood) disrupts thyroid hormone synthesis and produces changes in the thyroid gland consistent with goitrogenesis....The human data echo the results of the experimental animal findings in that the indications of an effect of resorcinol on thyroid function from case reports of individuals who applied copious amounts of resorcinol-containing skin ointments for periods of months to years.

However, CANTOX (2000) also concluded that epidemiological studies have not demonstrated an effect of resorcinol on the human thyroid due to workplace exposures. Also, they state that the results of their risk assessment “support the conclusion that under real world exposure conditions, resorcinol is not expected to cause adverse effects.”

Lynch et al. (2002) made similar conclusions in their publication of the same report as above. Accordingly, thyrotoxicity is defined as the critical effect for RfD derivation. Given that thyrotoxicity is the critical effect for resorcinol, there are a limited number of candidate studies to consider for the key study. Human case studies of individuals who received extremely high doses of resorcinol are inappropriate for RfD derivation. No properly designed and executed

human epidemiology studies exist that are suitable for RfD derivation. Acute or subacute animal studies are inappropriate for RfD derivation because their durations are too short, and the routes of exposure (peritoneal injection, subcutaneous injection, or dermal application) are not relevant to the derivation of an oral RfD. Two subchronic drinking water studies in rodents show adverse effects on the thyroid (Seffner et al., 1995 (84 days) and Cooksey et al., 1985 (30 days)), but both of these studies were classified as “use with care” by the European Commission summary on resorcinol.

The first of the two studies (Seffner et al., 1995) was not as useful as the WIL (2003) data for RfD derivation for several reasons: (1) no dose information was presented, (2) only a single drinking water concentration was tested, (3) no measurement of overt pathology or thyroid hormone levels was evaluated in this study, and (4) only changes in the size of thyroid cells were measured. The second (Cooksey et al., 1985) was not as useful as the WIL (2003) data because: (1) no information was provided on the concentration of resorcinol in water, (2) only a single drinking water concentration was tested, (3) thyroid weights were presented only graphically, and (4) no measurement of overt pathology or thyroid hormone levels was evaluated in this study, only measurement of incorporation of radioiodine after a 3-hour bolus dose.

Although no thyroid effects were seen in any animal group, the NTP subchronic and chronic studies also were not as useful as the WIL (2003) data for RfD derivation for three reasons: (1) dosing was gavage bolus and not continuous, which would be more relevant for RfD development, (2) circulating thyroid hormone levels were only measured for male and female rats in the 13-week study and (3) only T₃ and thyroxine were measured, not TSH.

The WIL (2003) study investigated thyroid toxicity in rats exposed to resorcinol by the oral route. While the NTP study was an oral study, administration of the aqueous solution of resorcinol was by gavage once a day. Rats in the WIL study were exposed to resorcinol on a continuous basis via their drinking water. The continuous drinking water route of administration more closely approximates the exposure route for humans, than the gavage route of exposure.

The WIL (2003) subchronic study has been chosen as the key study for thyrotoxicity. It reported no significant changes to thyroid hormone levels in male rats. An increase in total T₃ levels

occurred in F₀ females in the 360 mg/L group. However, since resorcinol acts by inhibiting peroxidase enzymes that results in lower T₃/thyroxine levels, the increase in T₃ levels is inconsistent with resorcinol's mode of action. Microscopic changes, i.e., follicular cell hyperplasia, were observed in the 360 mg/L group. The incidence of hyperplasia was not significantly different from control animals in any dose group. Thus, 360 mg/L, the highest dose tested, could be considered a NOAEL. However, while the incidence of follicular hyperplasia was not reported as statistically significant in the WIL (2003) report, the incidence does increase across doses, indicating a possible significant trend. AMEC performed alternative statistical tests to determine if these tests might show significance among the hyperplasia incidence data. The AMEC (2004) report is appended to this report.

The results of the additional analyses were inconclusive relative to identifying a LOAEL or a potential trend with dose when each animal group was analyzed separately due to the large confidence bounds that were calculated due to high variability in the experimental data. However, both a LOAEL and a dose-dependent trend were indicated when F₀ female and interim and scheduled necropsy F₀ male animals were grouped to yield larger sample sizes. Based on this analysis, AMEC concluded that the 360 mg/L dose can be designated as a LOAEL for thyroid hyperplasia. However, use of the 360 mg/L dose as a LOAEL is very conservative. Statistical significance was only shown when data from treatment groups were combined as noted above. However, there is uncertainty associated with merging data from male and female animals because of the potential gender specificity of resorcinol's effects on the thyroid, and AMEC believes that others reviewing the same data would conclude that 360 mg/L is a NOAEL for thyrotoxicity.

The drinking water concentrations reported in the WIL (2003) study when converted to mg/kg-day vary between males and females and vary for the different stages of the experiment as shown in Table 6. The average daily doses (ADD) for various time periods were reported in WIL (2003) with the exception of the doses during breeding. Water consumption during that period was not recorded (WIL, 2003). To estimate a dose for that period, AMEC averaged the "prior to" and "after" breeding doses in the case of the males, and averaged the "prior to" and "gestation" doses for females. The daily doses during lactation are greater than those doses during other phases of the study. This is due to an increase in water consumption during lactation.

Because the daily doses vary between the sexes and the phases of study, selecting a critical effect level or the Point of Departure (POD) is less straightforward than in cases where a single dose exists. As a first step in the selection process, AMEC calculated Average Daily Doses (ADDs) over the course of the study for interim necropsy F₀ males, scheduled necropsy F₀ males, and F₀ females (Table 7). In each case, the ADD equals the sum of the dose for each phase divided by the total number of days. For example, the total ADD for interim males in the 360 mg/l group equals [daily dose prior to breeding (36.9 mg/kg-day) x days prior to breeding (28 days) + daily dose during breeding (34.2 mg/kg-day) x days during breeding (14 days) + daily dose after breeding (31.4 mg/kg-day) x days after breeding (6 days)] /48 days or 35.4 mg/kg-day.

The next step in selecting the POD is to determine which total ADD from the 360 mg/L groups best represents the study. The most conservative approach would be to select 33.6 mg/kg-day, which is the lowest total ADD reported in either group of 360 mg/L males or in the 360 mg/L females. One could also consider the average of the two male groups (34.5 mg/kg-day) or the average of the two male groups and the females (43.3 mg/kg-day). The remaining option under consideration is the animal-weighted average of the two male groups and females. Based on seven male rats in both the interim and scheduled 360 mg/L group and 14 female rats, the weighted average equals 47.7 mg/kg-day.

As previously discussed, statistical significance for the 360 mg/L group was only shown when data from all animals (interim F₀ males, scheduled F₀ males and F₀ females) were combined. No significance was shown when comparisons were made between treated animals and controls within the individual groups. Therefore, it is reasonable to select the POD based on all animals. In line with this, the POD would be either the average of the three groups (43.3 mg/kg-day) or the weighted average (47.7 mg/kg-day). While the arithmetic average is more conservative, the animal-weighted average better represents the study. Accordingly, AMEC proposes 47.7 mg/kg-day as the POD. It is reasonable to consider this POD as either a NOAEL or as a LOAEL. However, as noted above, use of the 360 mg/L dose as a LOAEL is very conservative, and AMEC believes that others reviewing the same data would conclude that 47.7 mg/kg-day is a NOAEL for thyrotoxicity.

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 humans, they were primarily acute low-dose experiments for the purposes of obtaining pharmacokinetic data. Other information on humans, from case studies or epidemiology provides, at best, an estimate of no-effect levels. The key study used for the RfD evaluated multiple endpoints in rats, including thyroid toxicity.

Because of the similarity of pharmacokinetic data between experimental animals and humans, the fact that the critical endpoint is thyroid toxicity (also observed in humans) and the fact that resorcinol was administered in drinking water, which is optimal for characterization of human exposure, an extrapolation factor smaller than the default of 10 is appropriate. More importantly, it is known that the rat is more sensitive than humans to disruption in thyroid function (Hard, 1998; Capen, 2000), as the result of a substantially shorter half-life for circulating thyroxine (12-24 hours in rats *versus* 5-9 days in humans). The fact that rodents are more sensitive to the effect of thyroid disrupting agents than humans has been thoroughly discussed in a recent document issued by the European Commission entitled *Study on the Scientific Evaluation of 12 Substances in the Context of Endocrine Disrupter Priority List of Actions* (WRc-NSF, 2002). Specifically, WRc (2002) stated in the chapter on resorcinol: "It needs to be recognized that rodents, especially rats, have been reported to be particularly susceptible to goitrogens, primarily due to the lack of thyroid binding protein (TBP) which is the primary protein for thyroid hormone binding and transport....In the rat, the absence of TBP results in a much shorter half life of thyroxine and much higher levels of TSH....These differences suggest that the activity of the thyroid gland in rats is considerably higher than that of other species, including humans, and this increased activity correlates to a greater susceptibility to hormonally-induced thyroid effects....Given the relative insensitivity of humans to changes in the thyroid gland, it has been suggested by certain authors...that high doses of substances such as resorcinol which cause hormonally-induced changes of the thyroid in rodents (particularly rats) have limited

relevance to humans.” Accordingly, a value for UF_A of 1 is appropriate for the derivation of a RfD for resorcinol from the POD based on the thyroid effects in the WIL (2003) rat study.

6.2.2 Human Sensitivity (Intraspecies) Extrapolation (UF_H)

There are no studies identifying the range of sensitivities to resorcinol among humans. Thus, the adequacy of this uncertainty factor for protection of children was explicitly considered. As noted in Section 4.3 of this report, resorcinol is eliminated without phase I metabolism primarily as glucuronide conjugates. Neonates are well known to be underdeveloped with respect to this type of elimination process, such that increased sensitivity might be anticipated based on reduced clearance (Dorne et al., 2001; Ginsberg et al., 2002). However, conjugation mechanisms and clearance of conjugated compounds have also been shown to develop rapidly and to be equivalent to or more efficient than adult conjugation and clearance by only a few months of age (Dorne et al., 2001; Ginsberg et al., 2002). Ginsberg et al. (2002) reported that the ratio of the average child half-life to the average adult half-life for glucuronidation substrates in their database was 3 for neonates, 2 for infants 1 week to 2 months of age, 0.90 for infants 2-6 months of age, 1.1 for children 6-24 months of age and 1.2 for children 2-12 years of age. Dorne et al. (2001) reported that the ratio of the average child clearance rate to the average adult clearance rate for glucuronidation substrates in their database was 2.3 for neonates, 1.0 for infants, and 0.9 for children. Thus, the default pharmacokinetic uncertainty factor of 3.16 is adequate for resorcinol to protect average neonates who might be exposed early in life. For chronic exposures that occur after the neonatal period, the default factor of 3.16 is more than adequate. In addition, elderly people and people with impaired renal function may also have impaired conjugation rates. Dorne et al. (2001) reported that the ratio of the average elderly clearance rate to the average adult clearance rate for glucuronidation substrates in their database was 0.9 to 2.5 depending on the metric compared. The clearance rate ratios for adults with liver disease and renal disease were 1.0 to 2.1 and 1.5 to 2.5 respectively. Again, the default factor of 3.16 is adequate to protect average neonates, infants, children, elderly, and people with liver or kidney disease.

There are several case studies in the literature, which indicate that children may be particularly sensitive to certain acute toxic effects of resorcinol, such as methemoglobinemia. When Dorne et al. (2001) evaluated acute effects using the maximum plasma concentration (C_{max}) as the

pharmacokinetic metric for their comparisons, they found that the average ratios of C_{max} for neonates to healthy adults was 1.8, and the similar ratio for children was 0.8.

In addition, as noted above, the POD is based on either a NOAEL or a LOAEL for changes in thyroid hyperplasia, which were not accompanied by thyroid hypertrophy or changes in circulating thyroid hormones. The prevailing model for the mode of action for thyrotoxic chemicals is that alterations in circulating thyroid hormones (decreases in thyroxine and T_3 , increases in TSH) are early biological effects that precede altered thyroid structure, which includes thyroid cell hypertrophy followed by hyperplasia (Capen, 1997, 1998, 2000; Hard, 1998). No adverse effects on thyroid function were demonstrated. The observation defined as the POD (thyroid hyperplasia) could have been artifactual, given the fact that no differences were observed in circulating thyroid hormones compared to controls.

Accordingly, a value for UF_H of 3 is appropriate for the derivation of a RfD for resorcinol from the POD based on the thyroid hyperplasia in the WIL (2003) rat study to account for reduced clearance of resorcinol in children, the elderly, and the renally compromised.

6.2.3 LOAEL to NOAEL Extrapolation (UF_L)

The POD dose for thyroid hyperplasia as the critical effect has been interpreted as either a NOAEL or a LOAEL as discussed previously. Characterizing the dose level as a LOAEL is very conservative. Only after applying advanced statistical tests with all three groups of animals (male interim sacrifice group, male scheduled sacrifice group, and female scheduled sacrifice group) merged into a single data set was the 360 mg/L dose group found to be statistically significantly different from the control group. No significant differences were found in the hyperplasia incidence when individual animal groups were compared to controls. Due to the lack of response between groups, the 360 mg/L dose could be considered a NOAEL. Because of the very conservative nature of the LOAEL, a full uncertainty factor of 10 is not necessary. When the same weighted-average approach that was used to derive the LOAEL is applied to the next highest dose group (120 mg/L), the resulting NOAEL (16.2 mg/kg-day) is a factor of 3 less than the LOAEL (47.7 mg/kg-day). In other words, if one starts with the LOAEL and applies an UF_L of 3, the result is the same as if one starts with the NOAEL and applies no UF_L .

Therefore, a UF_L of 3 is appropriate if the POD is considered a LOAEL. If the POD is considered a NOAEL, then a UF_L of 1 is appropriate.

6.2.4 Subchronic to Chronic Extrapolation (UF_S)

The key study used for the RfD was a subchronic study. However, a default factor for UF_S of 10 is not required. Because resorcinol is cleared very quickly, and chronic studies do not show progression of effects, an UF_S of 3 is appropriate.

6.2.5 Database (UF_D)

The results of the key study (WIL, 2003) were largely consistent with studies conducted as part of the NTP subchronic and chronic resorcinol study program, as well as with 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.

The EPA requirement for a high quality database for RfD derivation includes two chronic animal bioassays by an appropriate route of exposure in two different species, one two-generation reproductive study, and two developmental studies in different species (EPA, 1993; Dourson, 1994.) The requirement for a minimal database is a single subchronic study. UF_D of 1 is used when the database quality is high and a UF_D of 3 is typically used when one or two of the required five studies is missing from the database. EPA rarely applies a UF_D of 10.

Specifically, the existing database includes two high quality subchronic studies by the oral route of exposure. These include the aqueous gavage studies in rats and mice (NTP, 1992) and the drinking water study in rats (WIL, 2003). The database also includes two high quality chronic studies by the oral route in rats and mice (NTP, 1992). There are also four high quality developmental studies by the oral route of exposure. Three were performed in rats (Hazelton, 1982a; DiNardo et al., 1985; Kavlock, 1990), and one was performed in rabbits (Hazelton,

1982b). Missing from the available literature is a properly designed and executed two-generation reproductive function study.

A multigenerational reproductive study has been conducted with resorcinol (Burnett and Goldenthal, 1988). It was, however, conducted using dermal application of the compound in combination with other compounds. It is thus not possible to determine if the doses were sufficiently high or applicable to the oral exposure route to have complete confidence in the negative findings.

Although no oral multigenerational reproductive study of resorcinol was found in the published literature, the WIL (2003) study reported above, which was a comprehensive dose range finding study using the oral exposure route with drinking water as the experimental medium, was one phase of a comprehensive two-generation reproductive toxicity study currently in progress. The WIL (2003) study examined reproductive performance in F₀ males and females, and developmental neurotoxicity in the F₁ offspring in this dose range finding study. Male and female mating and fertility indices were not statistically significant from controls. No other effects on reproduction were reported. The mean number of pups born, live litter size, and general physical condition of the F₁ offspring, including body weight were similar to control animals (WIL, 2003). Results of all neurobehavioral tests in the F₁ offspring were also similar to control animals. As previously reported, the statistical significance in locomotor activity reported in the study was caused by one or two outlier animals and since the increase in motor activity was the only neurobehavioral endpoint that was significant, resorcinol in this study did not cause neurotoxic effects.

In summary, the existing toxicological database contains four of the five required studies to be classified as a high quality database. The missing study is a two-generation reproductive study. Such a study is underway, and the key study defined in this document (WIL, 2003) is one phase of this two-generation study. The WIL (2003) dose range finding study examined thyrotoxicity, reproductive performance, late gestational and early neonatal toxicity, and developmental neurotoxicity. To account for the uncertainty that might still exist concerning the lack of the full two-generation study, an UF_D of 3 is appropriate. The database uncertainty factor may be decreased to 1 when the two-generation study is published.

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 or the LOAEL by the product of selected uncertainty factors. For resorcinol, the calculation is:

$$RfD = \frac{47.7 \text{ mg / kg - day}}{30} = 2 \text{ mg / kg - day}$$

where 47.7 mg/kg-day is the NOAEL for thyroid toxicity; a 3-fold factor each is applied for UF_H , UF_S , and UF_D , a 1-fold factor is applied for UF_A and UF_L , and the modifying factor is assigned a value of one.

Alternatively, the calculation is:

$$RfD = \frac{47.7 \text{ mg / kg - day}}{100} = 0.5 \text{ mg / kg - day}$$

where 47.7 mg/kg-day is the LOAEL for thyroid toxicity; a 3-fold factor each is applied for UF_H , UF_S , UF_L and UF_D , a 1-fold factor is applied for UF_A , and the modifying factor is assigned a value of one.

In conclusion, the RfD is 0.5 mg/kg-day if one conservatively considers the high dose in the WIL (2003) study a LOAEL for thyroid hyperplasia or 2 mg/kg-day if one considers the high dose in the WIL (2003) study a NOAEL for thyroid hyperplasia.

6.4 CONFIDENCE

The key study supporting the RfD evaluated many different endpoints including specific endpoints of interest, but it involved a limited number of animals, being a dose range finding study with a specific objective. Its objective was to identify doses of resorcinol that would cause minimal toxicity and yet allow for successful mating, gestation, parturition and lactation in a full two-generation reproductive study. As such, confidence in this study is medium. The overall database on toxicological effects of resorcinol including human studies is extensive and includes subchronic and chronic National Toxicology Program rat and mouse bioassays, but it does appear to have a data gap relating 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 System (IRIS, 2004). Also, there is uncertainty associated with merging data from male and female animals when performing statistical significance testing on thyrotoxicity data, because of the potential gender specificity of resorcinol's effects on the thyroid. Using the same narrative approach, overall confidence in the RfD is also medium. The uncertainty factors applied to derive the RfD are reflective of the medium confidence in the RfD database, such that the level of 0.5 to 2 mg/kg-day adequately meets the general definition of the RfD given in Section 1 and represents a dose without appreciable human risk of adverse effect for daily exposure over chronic exposure periods.

Based on its review of the available database, AMEC has concluded that there is adequate toxicity information available to support the derivation of a RfD at this time. However, AMEC acknowledges that the key study, while superior to other existing studies for RfD derivation, is a dose range finding study that was performed as a precursor to a guideline compliant two-generation reproductive study. In the future, when the full guideline compliant two-generation reproductive study has been completed, fully documented, and released to the public, AMEC may revise its RfD derivation to take into account the new information. The addition of the results of the full two-generation reproductive study using drinking water as the route of administration to the toxicological database will likely change the confidence in the database from medium to high.

AMEC notes that a RfD in the range of 0.5 to 2 mg/kg-day is similar to the RfD accepted in a site-specific human health risk assessment in the Commonwealth of Pennsylvania, which was

0.5 mg/kg-day. The Pennsylvania Department of Environmental Protection Southeast Regional Office accepted the value in a letter dated June 3, 2003 and signed by Bruce D. Beitler, Regional Manager. The letter refers to a document entitled "Resorcinol Release Investigation" prepared on February 26, 2003 for the Rohm and Haas Company by URS Corporation and submitted to the PADEP on March 5, 2003.

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Table 1. Summary of PubMed Resorcinol Literature Search

Classification	Number of citations
Total PubMed download	588
Citations found to be relevant to RfD development	74
Used as a component of a surgical and dental adhesive formula	126
Did not refer to resorcinol but involved a compound in which the resorcinol structure was a moiety of a larger molecule, for example 4-(2-pyridylazo) resorcinol	118
As a reagent for the detection of sialic acids for the quantitation of glycolipids	52
Analytical method papers that use resorcinol as a substrate to assess microbial degradation potential	30
Resorcinol in the investigation of chemical reactions, for example as a starting chemical in the synthesis of more complex molecules, or the interaction of resorcinol with other chemicals, e.g. polymerization reactions	28
Component of Weigert's resorcin-fuchsin reagent	18
Analytical method papers that investigate the separation and/or detection of resorcinol	17
Used in <i>in vitro</i> biochemical investigations for the ability of the molecule to attract divalent metals	17
Resorcinol as a phenolic compound in various <i>in vitro</i> tests	16
As a substrate for peroxidase enzymes	13
As a substrate for the <i>in vitro</i> investigation of hydroxylase activity	13
Antioxidant properties of resorcinol and other redox cycling phenolic compounds including the ability to scavenge reactive oxygen species	11
Resorcinol as a metabolite of phenol, 3-dinitrobenzene or other more complex molecules	11
Natural occurrences of resorcinol including resorcinol in humic substances, in sorghum plants, and in cigarette smoke	5
Role of resorcinol in the study was not clear from the abstract; it was clear that no toxicological information would be presented in paper	22
Inhibitory effects of catechol derivatives on arachidonic acid-induced aggregation of rabbit platelets	1
Tumor promotion for nitroso-compound induced cancer in rodents	1
Not evaluated because of publication language	15

**Table 2. Resorcinol Reference Dose
Summary of Human Studies**

Author	Study Type	Dose Route	Exposure Duration	Dose	Findings
Abbate et al., 1989	Clinical report on self-selected study group	occupational	not reported	not reported	dermatitis
Bontemps et al., 1995	Case Report	dermal/acne vulgaris	3 days	50% resorcinol in benzoin and zinc oxide	CNS stimulation, followed by cardiac and respiratory depression
Bull and Fraser, 1950	Case Report	dermal/ulcerated skin	6 months - 3 years	Case 2: 47 mg/kg-day(a); Case 3: 37 mg/kg-day(a)	thyroid dysfunction (myxoedema) in three case studies
Cunningham, 1956	Case Report	dermal/ damaged skin	1 day	12.5% ointment	methemoglobinemia, hemolytic anemia in a child treated for rash
Currie et al., 1997	Clinical report on self-selected study group	dermal	7 days	11.3 mg/kg-day(a)	no methemoglobinemia
Eierman et al., 1982	Prospective study of dermal reactions to cosmetics	dermal	variable	not reported	allergic dermatitis rare for resorcinol-containing products
Flickinger, 1976	Review of occupational data	occupational	not reported	not reported	no irritation or lost-time illness in plant workers
Gaitan, 1986, 1988; Gaitan et al., 1987/1988	Ecological epidemiology	ingestion	not reported	not reported	possible link of resorcinol in water to goiter
Goossens et al., 1999	Dermal reaction to cosmetics	dermal	not reported	not reported	1 case out of 475 allergic to resorcinol
Guerra et al., 1992a	Dermal reaction to cosmetics	dermal	not reported	not reported	1 case out of 261 allergic to resorcinol
Hart and MacLagan. 1951	Case Report	dermal/ulcerated skin	14 years	4% resorcinol in lanolin	thyroid dysfunction (myxoedema); goiter
Hobson, 1951	Case Report	dermal/ulcerated skin	11 months	12% resorcinol	thyroid dysfunction (myxoedema); goiter
Katin et al., 1977	Case Report	dermal	3 months	2.1 mg/kg-day(a)	hypothyroidism in patient on chronic hemodialysis
Lundell and Nordman, 1973	Case Report	dermal/ damaged skin	two applications	10% aqueous solution of resorcinol (Castellani's Solution)(b)	methemoglobinemia, hemolytic anemia in a child treated for rash
Lynch et al., 2002	Review of case and occupational epidemiology reports	dermal and occupational	variable	variable	no increased incidence of health effects in occupational cohorts; prolonged dermal exposure is thyrotoxic; estimated dermal no-effect dose for thyrotoxicity is 10 mg/kg-day.

**Table 2. Resorcinol Reference Dose
Summary of Human Studies**

Author	Study Type	Dose Route	Exposure Duration	Dose	Findings
Roberts et al., 1990	Occupational study (textile workers)	occupational	not reported	not reported	No cases of hypothyroidism. 4 cases with elevated TSH levels; 8 cases with elevated thyroid antibodies - all other measures of thyroid function normal. Individuals with elevated TSH levels and thyroid antibodies were designated as "unexposed"; however might have been exposed from ventilation outlets.
Tarvainen, 1995	Dermal reaction to cosmetics	dermal	7 years	patch test	4 cases out of 839 allergic to resorcinol
Thomas and Gisburn, 1961	Case Report	dermal/ulcerated skin	13 years	85 mg/kg-day(a)	thyroid dysfunction; goiter
TOMA, 1978	Occupational study - general health of workers	occupational	not reported	not reported	TSH levels slightly elevated in six out of 280 workers; T4 level slightly below normal range in one out of 280 workers. Two workers had palpable thyroid. No goiterous thyroids or signs of hypo- or hyperthyroidism in any workers.
TOMA, 1980	Occupational study - mortality study	occupational	average length of employment: 10 years	not reported	Overall mortality was 22% less than expected. No SMRs were significantly higher than the expected values.
TOMA, 1981	Occupational study - general health of workers	occupational	not reported	not reported	TSH and T4 levels for all 104 workers were within normal range
TOMA, 1982	Occupational study - general health of workers	occupational	not reported	not reported	TSH levels in 88 workers were within normal range; slightly elevated T4 levels in two workers; slightly depressed T4 level in one worker.
Tush and Kuhn, 1996	Case Report	dermal/diaper rash	1.5 days	108 mg/kg-day(a)	methemoglobinemia in a child treated for rash
Vilaplana et al., 1991	Case Report	dermal	repeated application in hair dye, 4 years	not reported	allergic dermatitis
West and Stafford, 1997	Occupational study (ordnance factory workers)	occupational	not reported	not reported	nonsignificant increase in odds ratios between blood abnormalities and resorcinol
Yeung et al., 1983	Clinical pharmacology and <i>in vitro</i> testing with human skin	dermal	6 days/week 4 weeks	12 mg/kg-day (dermal); 0.2 mg/kg-day (absorbed)(a)	estimate of dermal absorption rate, no effect on circulating thyroid hormone levels

notes:

(a) Doses estimated by the authors of this report, as described in the text

(b) Castellani's solution contains several other ingredients including 0.5% fuschin, 4% phenol, and 5% acetone.

Table 3. Resorcinol Reference Dose Summary of Animal Studies

Author	Species	Dose Route	Duration of Exposure	Dose (a)	Target	LOAEL (mg/kg-day)	NOAEL (mg/kg-day)
Hossack & Richardson, 1977 as cited in CIR, 1985	Rat	oral - gavage	2 doses, 24 hours apart	500 mg/kg-day	behavior	-	500
WIL, 2003	Rat	oral - drinking water	48 - 87days	10 - 360 mg/L	neurobehavioral changes	-	47.7
Flickinger, 1976	Rabbit	dermal, abraded skin	24 hours	1000-7950 mg/kg	body weight	1000	-
Flickinger, 1976	Rat	oral - gavage	single dose	398-3160 mg/kg	body weight	-	398
WIL, 2003	Rat	oral - drinking water	48 - 87 days	10 - 360 mg/L	body weight	-	47.7
Okazaki et al., 1993	Rat	oral - dietary	36 weeks	0.8% (715 mg/kg-day) (1)	body weight	715	-
Hazelton Labs. 1982a	Rat	oral - gavage	days 6-15 of gestation	40-250 mg/kg-day	body weight gain	-	250
Kavlock, 1990	Rat	oral - gavage	day 11 of gestation	333-1000 mg/kg	body weight gain	-	1000
Hazelton Labs. 1982b	Rabbit	oral - gavage	days 6-18 of gestation	25-100 mg/kg-day	body weight gain	100	-
NTP, 1992	Rat	oral - gavage	12 doses over 17 days	28-450 mg/kg-day	body weight	-	318
NTP, 1992	Mouse	oral - gavage	12 doses over 17 days	38-600 mg/kg-day	body weight	-	424
NTP, 1992	Rat	oral - gavage	5 days/week - 13 weeks	32-520 mg/kg-day	body weight	-	186
NTP, 1992	Mouse	oral - gavage	5 days/week - 13 weeks	28-420 mg/kg-day	body weight	-	300
NTP, 1992	Rat	oral - gavage	5 days/week - 103 weeks	50-150 mg/kg-day	body weight	-	107.0
NTP, 1992	Mouse	oral - gavage	5 days/week - 103 weeks	112-225 mg/kg-day	body weight	-	161
Burnett and Goldenthal, 1988 (also IRDC, 1979)	Rat	dermal	twice a week for 2 years	0.5-1 % solution (2)	cancer	-	82

Table 3. Resorcinol Reference Dose Summary of Animal Studies

Author	Species	Dose Route	Duration of Exposure	Dose (a)	Target	LOAEL (mg/kg-day)	NOAEL (mg/kg-day)
Stenback and Shubik, 1974	Mouse	dermal	twice a week for 75 weeks	5 - 50 % solution (3)	cancer	-	95
Stenback, 1977	Rabbit	dermal	twice a week for 160 weeks	5 - 50 % solution (4)	cancer	-	0.6
Bracher et al., 1981	Rat	dermal (shaved skin)	delivered in up to 3 doses (20 minutes each) in a period of 24 hours	0.2-100 mg/kg	chromosomes	-	100
Bracher et al., 1981	Rat	intraperitoneal	single dose	1-100 mg/kg	chromosomes	-	100
Bracher et al., 1981	Rat	oral - gavage	single dose	0.8-100 mg/kg	chromosomes	-	100
WIL, 2003	Rat	oral - drinking water	48 - 87 days	10 - 360 mg/L	CNS	-	47.7
Merker et al., 1982	Rat	subcutaneous	single dose	55-350 mg/kg	CNS	140	88
NTP, 1992	Rat	oral - gavage	12 doses over 17 days	28-450 mg/kg-day	CNS	39	19
NTP, 1992	Mouse	oral - gavage	12 doses over 17 days	38-600 mg/kg-day	CNS	106	53
NTP, 1992	Rat	oral - gavage	5 days/week - 13 weeks	32-520 mg/kg-day	CNS	371	186
NTP, 1992	Mouse	oral - gavage	5 days/week - 13 weeks	28-420 mg/kg-day	CNS	300	161
NTP, 1992	Rat	oral - gavage	5 days/week - 103 weeks	50-150 mg/kg-day	CNS	71.4	35.7
NTP, 1992	Mouse	oral - gavage	5 days/week - 103 weeks	112-225 mg/kg-day	CNS	80	-
NTP, 1992	Mouse	oral - gavage	5 days/week - 13 weeks	28-420 mg/kg-day	clinical chemistry, hematology	-	300
NTP, 1992	Mouse	oral - gavage	5 days/week - 60 week (interim evaluation)	112-225 mg/kg-day	clinical chemistry	-	161
Flickinger, 1976	Rat	oral - gavage	single dose	398-3160 mg/kg	mortality	795	398

Table 3. Resorcinol Reference Dose Summary of Animal Studies

Author	Species	Dose Route	Duration of Exposure	Dose (a)	Target	LOAEL (mg/kg-day)	NOAEL (mg/kg-day)
Hazelton Labs. 1982a	Rat	oral - gavage	days 6-15 of gestation.	40-250 mg/kg-day	mortality	-	250
WIL, 2003	Rat	oral - drinking water	48 - 87 days	10 - 360 mg/L	mortality	-	47.7
NTP, 1992	Rat	oral - gavage	12 doses over 17 days	28-450 mg/kg-day	mortality	-	318
NTP, 1992	Mouse	oral - gavage	12 doses over 17 days	38-600 mg/kg-day	mortality	212	106
NTP, 1992	Rat	oral - gavage	5 days/week - 13 weeks	32-520 mg/kg-day	mortality	371	186
NTP, 1992	Mouse	oral - gavage	5 days/week - 13 weeks	28-420 mg/kg-day	mortality	300	161
NTP, 1992	Rat	oral - gavage	5 days/week - 103 weeks	50-150 mg/kg-day	mortality	107	71
NTP, 1992	Mouse	oral - gavage	5 days/week - 103 weeks	112-225 mg/kg-day	mortality	-	161
Flickinger, 1976	Rabbit	dermal, abraded skin	24 hours	1000-7950 mg/kg	mortality	2000	1000
Stenback and Shubik, 1974	Mouse	dermal	75 weeks	5 - 50 % solution	Organ toxicity	-	95.2
Okazaki et al., 1993	Rat	oral - dietary	36 weeks	0.8% (715 mg/kg-day) (1)	increased liver and kidney weights	715	-
FEMA, 1979 as cited in CIR, 1985	Rat	oral - dietary	4 weeks	261 mg/kg-day	adrenal weight (decreased adrenal : BW ratio)	261	-
WIL, 2003	Rat	oral - drinking water	48 - 87 days	10 - 360 mg/L	adrenal weight	-	47.7
NTP, 1992	Rat	oral - gavage	12 doses over 17 days	28-450 mg/kg-day	thymus weight	318	159
NTP, 1992	Mouse	oral - gavage	12 doses over 17 days	38-600 mg/kg-day	organ weight - no changes	-	212
NTP, 1992	Rat	oral - gavage	5 days/week - 13 weeks	32-520 mg/kg-day	increased liver, adrenal weight	22.9	-

Table 3. Resorcinol Reference Dose Summary of Animal Studies

Author	Species	Dose Route	Duration of Exposure	Dose (a)	Target	LOAEL (mg/kg-day)	NOAEL (mg/kg-day)
NTP, 1992	Mouse	oral - gavage	5 days/week - 13 weeks	28-420 mg/kg-day	adrenal weight	20	-
NTP, 1992	Mouse	oral - gavage	5 days/week - 60 week (interim evaluation)	112-225 mg/kg-day	organ weight - no changes at 15 month evaluation	-	161
NTP, 1992	Rat	oral - gavage	5 days/week - 60 week (interim evaluation)	112-225 mg/kg-day	increased relative brain weight males	80	-
NTP, 1992	Rat	oral - gavage	5 days/week - 60 week (interim evaluation)	50-150 mg/kg-day	increased relative liver weight females	107	71
WIL, 2003	Rat	oral - drinking water	48 - 87 days	10 - 360 mg/L	reproductive outcome	-	47.7
DiNardo et al., 1985	Rat	oral - gavage	days 6-15 of gestation.	125-500 mg/kg-day	reproductive outcome	-	500
Kavlock, 1990	Rat	oral - gavage	day 11 of gestation	333-1000 mg/kg	reproductive outcome	-	1000
Flickinger, 1976	Rabbit	dermal, abraded skin	24 hours	1000-7950 mg/kg	skin	2000	1000
NTP, 1992	Rat	oral - gavage	5 days/week - 13 weeks	32-520 mg/kg-day	thyroid		186
NTP, 1992	Mouse	oral - gavage	5 days/week - 13 weeks	28-420 mg/kg-day	thyroid		161
NTP, 1992	Rat	oral - gavage	5 days/week - 103 weeks	50-225 mg/kg-day	thyroid		71
NTP, 1992	Mouse	oral - gavage	5 days/week - 103 weeks	112-225 mg/kg-day	thyroid		161
Arnott and Doniach, 1952	Rat	subcutaneous	single dose	40-180 mg/kg	thyroid	70	-
Berthezene et al., 1979	Rat	oral - dietary	2 weeks	5% (4468 mg/kg/day) (5)	thyroid	4468	-
WIL, 2003	Rat	oral - drinking water	48 - 87 days	10 - 360 mg/L	thyroid	47.7	16.2
Cooksey, et al. 1985	Rat	oral - drinking water	30 days	10 mg/kg-day	thyroid	10	-

Table 3. Resorcinol Reference Dose Summary of Animal Studies

Author	Species	Dose Route	Duration of Exposure	Dose (a)	Target	LOAEL (mg/kg-day)	NOAEL (mg/kg-day)
Klein et al., 1956	Rabbit	subcutaneous	daily for 19 days	50-75 mg/kg-day	thyroid	-	75
Samuel, 1955	Rat	subcutaneous	twice a day for up to 79 days	396 mg/kg-day	thyroid	396	-
Samuel, 1955	Rat	dermal, scarified skin	10 minutes, twice a day, for 28 days	12.5% ointment	thyroid	40,000	-
Seffner, et al., 1995	Rat	oral - drinking water	84 days	40 mg/L (6 mg/kg-day)	thyroid	6	-

notes:

(a) "Doses" are given in the units described in the reports and may not be comparable between experiments. Certain doses have been estimated or converted to units of mg resorcinol per kilogram body weight (mg/kg) by the authors of this RfD report. Doses were adjusted for exposure duration if a daily dosage regime was not used.

1. Rat body weight assumed to be 235g (TERA, 2002). Rat food intake assumed to be 21 g/day (TERA, 2002). At the food concentration of 0.8% resorcinol, daily dose = $21 \text{ g food/day} \times 0.8\% \text{ resorcinol} / 100\% \text{ food} / 0.235 \text{ kg BW} \times 1000 \text{ mg/g} = 715 \text{ mg/kg day}$
2. Daily dermal dose calculated by multiplying the applied concentration by the applied volume and adjusting for the twice a week dosing regime and duration of exposure and animal body weight. e.g. Daily dose = 1% solution = $1 \text{ g}/100 \text{ ml} \times 0.5 \text{ ml application volume} = 0.1 \text{ g}$ applied twice a week for 104 weeks i.e. 208 days applied over 728 days = $0.1 \text{ g} \times 208 / 728 = 0.02857 \text{ g/day} / 0.350 \text{ kg BW} \times 1000 \text{ mg/g} = 81.6 \text{ mg/kg-day}$
3. Daily dermal dose calculated by multiplying the applied concentration by the applied volume and adjusting for the twice a week dosing regime and duration of exposure and animal body weight. e.g. Daily dose = 50% solution = $50 \text{ g}/100 \text{ ml} \times 0.02 \text{ ml application volume} = 0.01 \text{ g}$ applied twice a week for 100 weeks i.e. 200 days applied over 700 days = $0.002 \text{ g} \times 200 / 700 = 0.00286 \text{ g/day} / 0.03 \text{ kg BW} \times 1000 \text{ mg/g} = 95.2 \text{ mg/kg-day}$
4. Daily dermal dose calculated by multiplying the applied concentration by the applied volume and adjusting for the twice a week dosing regime and duration of exposure and animal body weight. e.g. Daily dose = 50% solution = $50 \text{ g}/100 \text{ ml} \times 0.02 \text{ ml application volume} = 0.01 \text{ g}$ applied twice a week for 160 weeks i.e. 320 days applied over 1120 days = $0.01\text{g} \times 320 / 1120 = 0.002857 \text{ g/day} / 4.755 \text{ kg BW} \times 1000 \text{ mg/g} = 0.601 \text{ mg/kg-day}$
5. Rat body weight assumed to be 235g (TERA, 2002). Rat food intake assumed to be 21 g/day (TERA, 2002). At the food concentration of 5% resorcinol, daily dose = $21 \text{ g food/day} \times 5\% \text{ resorcinol} / 100\% \text{ food} / 0.235 \text{ kg BW} \times 1000 \text{ mg/g} = 4468 \text{ mg/kg day}$

**Table 4. Resorcinol Reference Dose
National Toxicology Program Studies
Selected Findings in 91-Day Dosing Study**

Dose mg/kg-day	survival	final body	necropsy body	liver		adrenal	adrenal/bw	kidney	kidney/bw	brain	brain/bw	qualitative CNS effects ^b
		weight ^a (grams)	weight ^a (grams)	weight (grams)	liver/bw ratio	weight (milligrams)	ratio	weight (grams)	ratio	weight (grams)	ratio	
female mice												
0	10/10	24	20.5	0.98	0.0480	9.10	0.00044	0.17	0.00826	0.44	0.0216	no
28	10/10	23.8 (99)	19.7 (96)	0.92	0.0466	8.11	0.00041	0.16	0.00837	0.45	0.0227	no
56	10/10	24.2 (101)	20 (98)	0.98	0.0488	8.09	0.00041	0.17	0.00839	0.43	0.0215	no
112	10/10	24.3 (101)	20.3 (99)	0.93	0.0459	10.20	0.0005	0.16	0.00810	0.44	0.0215	no
225	10/10	24.2 (101)	20.3 (99)	0.94	0.0464	10.60	0.00052	0.16	0.00779	0.43	0.0212	no
420	2/10	23.5 (98)	19.5 (95)	1.00	0.0512	9.50	0.00049	0.18	0.00936	0.45	0.0231	yes
female rat												
0	10/10	202	183	4.77	0.0260	5.70	0.000031	0.66	0.00361	1.64	0.00897	no
32	10/10	206 (102)	182 (99)	5.15	0.0283	5.87	0.000032	0.66	0.00362	1.64	0.00902	no
65	10/10	203 (100)	183 (100)	5.43	0.0297	5.88	0.000032	0.68	0.00369	1.64	0.00899	no
130	10/10	206 (102)	187 (102)	5.41	0.0288	5.69	0.000031	0.70	0.00374	1.66	0.00887	no
260	6/10	201 (99)	182 (99)	5.49	0.0320	5.88	0.000032	0.70	0.00384	1.67	0.00918	no
520	0/10											yes

notes

values shown in bold italics are statistically different from controls (at least the p<0.05 level); statistical inference on survival not conducted.

(a) values in parentheses are per cent of control

(b) hyperexcitability, tachypnea, recumbency, and tremors were interpreted by NTP (1992) as acute resorcinol effects

**Table 4. Resorcinol Reference Dose
National Toxicology Program Studies
Selected Findings in 91-Day Dosing Study**

	survival	final body weight ^a (grams)	necropsy body weight ^a (grams)	liver weight (grams)	liver/bw ratio	adrenal weight (milligrams)	adrenal/bw ratio	kidney weight (grams)	kidney/bw ratio	brain weight (grams)	brain/bw ratio	qualitative CNS effects ^b
male mice												
Dose mg/kg-day												
0	10/10	32.4	27.6	1.18	0.0429	8.30	0.000301	0.25	0.00909	0.42	0.0153	no
28	10/10	30.7 (95)	25.3 (92)	1.19	0.0469	6.40	0.000250	0.26	0.01013	0.42	0.0167	no
56	10/10	31.6 (98)	26.4 (96)	1.2	0.0457	5.90	0.000220	0.24	0.00911	0.42	0.0160	no
112	9/10	31 (96)	25.3 (92)	1.16	0.0458	5.89	0.000230	0.23	0.00917	0.43	0.0170	no
225	10/10	30.3 (94)	25.3 (96)	1.12	0.0442	5.70	0.000230	0.24	0.00937	0.42	0.0165	no
420	2/10	29.5 ^c (91)	24 (87)	1.08	0.0451	9.00	0.000360 ^d	0.23	0.00954	0.43	0.0178	yes
male rat												
0	10/10	361	338	10.84	0.0320	4.73	0.000014	1.17	0.00345	1.83	0.005410	no
32	10/10	358 (99)	338 (100)	11.36	0.0336	5.42	0.000016	1.15	0.00343	1.82	0.005390	no
65	10/10	362 (100)	342 (101)	11.32	0.0331	5.48	0.000016	1.18	0.00344	1.82	0.005350	no
130	10/10	362 (100)	341(101)	11.75	0.0344	5.21	0.000016	1.21	0.00355	1.83	0.005360	no
260	8/10	365 (101)	337 (100)	11.74	0.0349	5.74	0.000015	1.23	0.00364	1.80	0.005360	no
520^e	2/10	358 (99)										yes

notes

values shown in bold italics are statistically different from controls (at least the p<0.05 level); statistical inference on survival not conducted.

(a) values in parentheses are per cent of control

(b) hyperexcitability, tachypnea, recumbency, and tremors were interpreted by NTP (1992) as acute resorcinol effects.

(c) final body weight in the 420 mg/day group in not significantly different, but weight gain during the course of dosing was statistically lower than controls

(d) only one adrenal gland was weighed in this group. No statistical procedure was possible.

(e) no organs were weighed in the 520 mg/kg day group

**Table 5. Resorcinol Reference Dose
National Toxicology Program Studies
Selected Findings in 2-Year Dosing Study**

	In-Life Observations	Interim Sacrifice (15 months; n= 10)					Terminal Results			
	qualitative CNS effects ^a	necropsy body weight ^b (grams)	brain weight (grams)	liver weight brain/bw ratio	liver weight (grams)	liver/bw ratio	final body weight ^b (grams)	survival at 109 weeks	mean survival (days)	
		female mouse								
Dose mg/kg-day										
0		28.1	0.46	0.0166	1.21	0.0434	38.2	38/60	654	
112	yes	29.2 (104)	0.48	0.0171	1.29	0.0448	36.5 (96)	33/60	621	
225	yes	27.5 (98)	0.46	0.0168	1.23	0.0450	34 ^c (89)	34/60	627	
		female rat								
0		291	1.90	0.00660	8.55	0.00295	351	34/60	658	
50	no	293 (101)	1.89	0.00648	8.55	0.00292	342 (97)	33/60	653	
100	yes	304 (104)	1.85	0.00609	9.26	0.00305	326 (93)	28/60	646	
150	yes	274 (94)	1.90	0.00695	8.72	0.00319	303 ^c (86)	24/60	595	

notes

values shown in bold italics are statistically different from controls (at least the p<0.05 level); statistical inference on survival ratio not conducted

(a) hyperexcitability, tachypnea, recumbency, and tremors were interpreted by NTP (1992) as acute resorcinol effects

(b) values in parentheses are per cent of control

(c) considered sufficient weight difference to constitute maximally tolerated dose

**Table 5. Resorcinol Reference Dose
National Toxicology Program Studies
Selected Findings in 2-Year Dosing Study**

	In-Life Observations	Interim Sacrifice (15 months; n= 10)					Terminal Results		
	qualitative CNS effects ^a	necropsy body weight ^b (grams)	brain weight (grams)	brain/bw ratio	liver weight (grams)	liver/bw ratio	final body weight ^b (grams)	survival at 109 weeks	mean survival (days)
Dose mg/kg-day		male mouse							
0		30.3	0.45	0.0150	1.38	0.0459	36.2	37/60	664
112	yes	31.1 (103)	0.44	0.0142	1.34	0.0433	34.3 (95)	43/60	678
225	yes	31.4 (104)	0.45	0.0143	1.33	0.0424	35.5 (98)	34/60	591
		male rat							
0		421	2.00	0.00475	12.69	0.00301	428	28/60	631
112	yes	393 (93)	2.04	0.00519	12.8	0.00326	421 (98)	25/60	629
225^d	yes						363 ^c (85)	9/60	472

notes

values shown in bold italics are statistically different from controls (at least the p<0.05 level); statistical inference on survival ratio not conducted.

(a) hyperexcitability, tachypnea, recumbency, and tremors were interpreted by NTP (1992) as acute resorcinol effects

(b) values in parentheses are per cent of control

(c) considered sufficient weight difference to constitute maximally tolerated dose

(d) no interim sacrifice was conducted in 225 mg/kg day dose due to high early mortality.

Table 6. Mean Calculated Compound Consumption (mg/kg-day)

Drinking Water Concentrations	Males			Females			
	Prior to Breeding ^a	During Breeding ^b	After Breeding ^a	Prior to Breeding ^a	During Breeding ^b	Gestation ^a	Lactation ^a
0 mg/L	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10 mg/L	0.8	0.7	0.6	0.8	1.1	1.3	2.9
40 mg/L	3.9	3.4	2.8	5.1	5.1	5.1	11.8
120 mg/L	13.1	11.8	10.5	15.6	15.1	14.6	34.1
360 mg/L	36.9	34.2	31.4	46.6	45.3	44.0	100.5

a: as reported in WIL (2003)

b: average of "prior to" and "after" breeding doses for males; average of "prior to" and "gestation" doses for females

Table 7. Average Daily Doses Over Entire Study

	Daily Dose Prior to Breeding (mg/kg-day)	Days Prior to Breeding (days)	Daily Dose During Breeding (mg/kg-day)	Days of Breeding (days)	Daily Dose After Breeding (mg/kg-day)	Days After Breeding (days)	Daily Dose (mg/kg-day)	Total Dose (mg/kg)
<i>Interim Necropsy F₀ Males</i>								
10 mg/L	0.8	28	0.7	14	0.6	6	0.75	36
40 mg/L	3.9	28	3.4	14	2.8	6	3.6	174
120 mg/L	13.1	28	11.8	14	10.5	6	12.4	595
360 mg/L	36.9	28	34.2	14	31.4	6	35.4	1700
<i>Scheduled Necropsy F₀</i>								
10 mg/L	0.8	28	0.7	14	0.6	45	0.7	59
40 mg/L	3.9	28	3.4	14	2.8	45	3.3	283
120 mg/L	13.1	28	11.8	14	10.5	45	11.5	1005
360 mg/L	36.9	28	34.2	14	31.4	45	33.6	2925

	Daily Dose Prior to Breeding (mg/kg-day)	Days Prior to Breeding (days)	Daily Dose During Breeding (mg/kg-day)	Average Days of Breeding (days)	Daily Dose During Gestation (mg/kg-day)	Days of Gestation (days)	Daily Dose During Lactation (mg/kg-day)	Days of Lactation (days)	Daily Dose (mg/kg- day)	Total Dose (mg/kg)
<i>F₀ Females</i>										
10 mg/L	0.8	28	1.1	6	1.3	20	2.9	21	1.5	116
40 mg/L	5.1	28	5.1	6	5.1	20	11.8	21	7.0	523
120 mg/L	15.6	28	15.1	6	14.6	20	34.1	21	20.5	1536
360 mg/L	46.6	28	45.3	6	44	20	100.5	21	60.9	4567

ATTACHMENT A

RESORCINOL TASKS:

LOCOMOTOR ACTIVITY AND THYROID HYPERPLASIA

**RESORCINOL TASKS:
LOCOMOTOR ACTIVITY AND THYROID HYPERPLASIA**

Submitted to:

Beazer East, Inc.

Submitted by:

AMEC Earth & Environmental
Portland, ME

September, 2004

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1.0 INTRODUCTION

As part of a recent study entitled “A Drinking Water Dose Range-Finding Reproductive Toxicity Study of Resorcinol in Rats, performed by WIL Research Lab and sponsored by the Resorcinol Task Force (WIL, 2003), animals were randomly assigned to several different sensory function and behavioral tests, including acoustic startle response, locomotor activity, functional observational battery and learning and memory tests. Among the various sensory and behavioral tests conducted, statistically significant effects were only observed for the locomotor activity test. Locomotor activity increased in a dose-responsive fashion in males at post-natal day (PND) 61. Activity was not significantly increased in females at PND 61 or at PND 21 in either males or females. The report concluded, “In the context of a dose range-finding study of limited power, the numerical increases in motor activity were not considered as conclusive evidence of a change in CNS status.”

AMEC was asked to determine whether the statistically significant effects observed for locomotor activity represent biologically significant effects. Part I of this report summarizes the results of this analysis. In addition to looking for evidence that supports the lack of biological significance of an increase in locomotor activity, AMEC investigated the statistical analyses that were used in the WIL (2003) report to determine the statistical significance of locomotor activity. The purpose of this task was to answer two questions: 1) Were the statistical tests employed appropriate? and 2) Are there other appropriate tests, that, when applied, do not result in a statistical significance? Part II of this report discusses the results of this analysis. Part II also summarizes the results of our investigation of the statistical analyses used to measure the significance of follicular hyperplasia. While the incidence of follicular hyperplasia was not reported as significant in the WIL (2003) report, the incidence increases across doses, indicating a possible significant trend. AMEC performed alternative statistical tests to determine if these tests might show significance among the hyperplasia incidence data.

2.0 PART I: SIGNIFICANCE OF INCREASE IN LOCOMOTOR ACTIVITY

While the WIL (2003) study's conclusion seems reasonable, AMEC examined the available literature to determine if a finding of increased locomotor activity in the absence of other CNS findings is without adverse effect. We searched the literature to find 1) information on how the locomotor test ranks when compared to other neurotoxicity tests, 2) studies that discuss its possible biological relevance and 3) studies that discuss if changes in locomotor activity have been observed when other known thyrotoxic chemicals were tested. Studies were available on motor activity tests (locomotor activity and motor activity are used interchangeably throughout this document). However, we were not successful in locating information on locomotor activity for other known thyrotoxic chemicals. As an alternative, we looked for precedent-setting cases where changes in motor activity were reported, but such changes were considered to be of no consequence.

2.1 LITERATURE SEARCH

The specific keyword searches performed for the literature search included neurotoxin, neurotoxicity, neurotoxicity testing methods, sensory function, acoustic startle response, motor activity and locomotor activity. The databases searched included NIEHS, TOXNET, EPA, GOOGLE, URSUS, AGRICOLA, ILSI and the Maine Library System. We reviewed over 30 articles related to the use of the motor activity endpoint in neurotoxicity testing and determined that there is an extensive dialogue in the scientific community with regard to the significance of changes in motor activity observed during neurotoxicity testing.

2.2 RESULTS

When evaluating a chemical for neurotoxicity, a tiered approach is generally used (National Research Council (NRC), 1992; Tilson, 1993, 2000; Kulig, 1996). The first tier consists of simple and rapid tests that are designed to assess a range of sensorimotor functions. These first-tier screening tests include functional observational batteries (FOB) and motor activity tests. Second-tier tests, which are more sensitive and more costly, are designed to characterize effects on sensory, motor and cognitive function and often are used to determine mechanism of

action or establish a No Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL). The FOB is a group of noninvasive tests used to evaluate neurologic and behavioral signs of exposed animals and to screen chemicals for further neurotoxicity testing (Office of Technology Assessment (OTA), 1990; Kulig 1996). In view of the fact that there were no significant changes in the FOB results as reported in the WIL (2003) study, our analysis focused only on the significance of changes in motor activity.

EPA (1998a,b) defines motor activity as "any movement of the experimental animal." Motor activity encompasses an array of behaviors that "involve coordinated participation of sensory, motor and integrative processes" (MacPhail et al., 1989). Motor activity is commonly measured by an automated activity recording apparatus that detects both increases and decreases in movement of the experimental animal. In the WIL (2003) study, the locomotor activity was measured using a San Diego Instruments (SDI) Photobeam Activity System. This device utilizes a series of infrared photobeams that track the activity of a test subject by measuring the interruption of the photobeams over a period of time. Locomotor activity was measured as total, fine, and ambulatory activity counts. The WIL (2003) results indicated that there was a statistically significant difference in the total and ambulatory activity of males in the 40, 120 and 360 mg/L dose groups as the animals approached sexual maturity.

While motor activity tests are reliable screening tools, there is consensus that a single measure of activity is not sufficient evidence for neurotoxicity (Reiter, 1978; Gerber and O'Shaughnessy, 1986; Sette, 1987; Maurissen and Mattsson, 1989; Mullenix, 1989; OTA, 1990; Moser, 2000). Because of this, motor activity tests are not employed as exclusive methods of neurotoxicity testing; rather they are applied in conjunction with other central nervous system tests (e.g., Functional Observational Batteries). OTA (1990) reports: "There is general agreement within the scientific community that questions remain concerning the specificity of motor activity measures." The EPA Guidelines for Developmental Neurotoxicity Risk Assessment (1991) indicate that it is important to be aware that with the number of endpoints that can be observed in standard protocols for developmental toxicity studies, a few statistically significant results may occur by chance. Furthermore, in EPA's Guidelines for Neurotoxicity Risk Assessment (1998), EPA states that screening studies based on simple observations involving autonomic and motor function observations would constitute "insufficient evidence" to classify a chemical as neurotoxic.

According to Mullenix (1989), the purpose of an activity test must be more than just the detection of hyper- or hypoactivity, terms well known to be of limited meaning. Furthermore, Mullenix (1989) reports:

“The evolution of activity tests is not complete. The most important step, to correlate a specific hyperactivity with defined pathology, remains to be achieved. A single measure of activity cannot be used to distinguish hyperactivities induced by very different mechanisms and associated with different long-term clinical consequences.”

Furthermore, Mullenix (1989) asserts: “I would not rely on one statistically significant change in frequency of one behavior to assign the label “adverse” effect.” Maurissen and Mattsson (1989) state: “When an increase or decrease in motor activity is noted, its significance in terms of an adverse event must be evaluated. In a test situation where no strict contingencies of reinforcement are stipulated (e.g., motor activity testing), behavioral changes cannot be interpreted *ipso facto* as adverse. Sette (1987) states: “Behavioral effects are not always neurotoxic or adverse... While neurotoxicity implies a toxic effect on the nervous system, behavioral effect is more a general term and points toward a behavioral change, whether this effect originates in the nervous system or not. When neurobehavioral techniques generate data that do not reflect nervous system involvement, these data, no matter how legitimate, are not relevant to evaluation of neurotoxicity.” In addition, WHO (1986) states:

“Considerable controversy exists concerning what constitutes an adverse effect in toxicology. According to one view, any evidence of a behavioural or biological change is considered to be an adverse effect. According to others, evidence is required of both an irreversible decrement in the ability of the organism to maintain homeostasis and/or an enhanced susceptibility to the deleterious effects of other environmental influences. In this latter view, differentiation between “nonadversive” and “adverse” effects requires considerable knowledge of the importance of reversible changes and subtle departures from “normal” behaviour, physiology, biochemistry, and morphology in terms of the organism's overall economy of life, ability to adapt to other stresses, and their possible effects on life span. Real or potential risks to the nervous system are difficult to assess because of its complexity. Some of the problems in assessment are associated with the wide variations that can occur but are still considered to be within the “normal” range. Some are associated with the plasticity of the nervous system. Other problems in

assessment are related to incomplete understanding of what is being measured by certain tests. It is clear, therefore, that no single test will suffice to examine the functional capacity of the nervous system.”

The use of activity as a unitary behavior should be discouraged (Reiter, 1978). The specific mechanism that causes a change in activity is difficult to pinpoint when the phenomena being measured are a network of several mechanisms. Because of the multifaceted nature of motor activity, to postulate that one single source caused an overall change in a network of behaviors would be conjecture (Maurissen and Mattsson, 1989). The EPA (1991) states: "A great deal of scientific judgment based on experience with developmental toxicity data and with principles of experimental design and statistical analysis may be required to adequately evaluate such data."

When testing motor activity, EPA (1985) guidelines require that the test and control groups “be designed to contain a sufficient number of animals at the completion of the study to detect a 40 percent change in activity of the test groups relative to the control group with 90 percent power at the 5 percent level.” Mean motor activity counts for PND 61 male rats are summarized in Table 92 of the WIL (2003) report. The mean for total activity counts in controls was reported at 1599. A 40% increase in activity would be approximately 2239 counts. The mean total counts for the 40 and 120 mg/l groups were 2141 and 2215, respectively. While these counts were reported as significantly different from controls at the $p=0.05$ level, they do not represent a 40% change as recommended by EPA (1985). The mean total activity count for the 360 mg/L group was 2254. This change was reported as significantly different from controls at the $p=0.01$ level and does slightly exceed the 40% change. The mean for ambulatory activity counts in controls was reported at 513. A 40% increase in activity would be approximately 718. The mean ambulatory counts for the 120 and 360 mg/L were 769 and 787, respectively. These counts were reported as significantly different from controls at the $p=0.01$ level and represent a slightly greater than 50% change.

However, even if motor activity changes are greater than 40%, such changes do not necessarily indicate the presence of adverse effects. For instance, Mullenix (1998) states:

“Simplicity is convenient, but armed only with this one dimensional measure of something as diverse as spontaneous behavior can lead to vulnerability in certain unavoidable situations: for example, if a 40% change in activity occurs, a description of

the behavioral manifestations is impossible. Clinical hyperactivity in children gives a false impression of increased motor act frequency, while in reality it consists of “disorganized” behavior. How well can interruptions of photocell beams describe disorganization? Also, if a 40% change in activity is induced by two substances, an indication that they act via different mechanisms must be gained from other tests. Ranking substances by their neurotoxic potential would be all but impossible except in extreme cases.”

In this quotation, Mullenix (1998) is stating that increased locomotor activity as measured by an increased number of interruptions of the photocell beams does not define the presence of an adverse effect, in this case clinical hyperactivity.

Because motor activity is an apical test (Reiter and MacPhail, 1979; MacPhail et al., 1989), it can be altered by a number of factors affecting the physiology of living organisms (Reiter, 1978; Gerber and O’Shaughnessy, 1986; Maurissen and Mattsson, 1989; US Office of Technology Assessment (OTA), 1990; Tilson, 1993). Gerber and O’Shaughnessy (1986) maintain that a behaviorally neurotoxic effect can be determined only when behavioral changes occur in the absence of or prior to demonstrable impairment of non-neural functions. Similarly, Maurissen and Mattsson (1989) state, “Only in the absence of systemic toxicity can a dose-related change in motor activity reflect an effect on the nervous system. Thus, statements about nervous system involvement can in fact only be made by exclusion, and the likelihood of a nervous system effect increases as confounders from other potential etiologies are ruled out.” The Office of Technology Assessment (OTA, 1990) asserts, “There is disagreement as to whether motor activity is a primary indicator of neurotoxicity. For example, the primary action of a toxicant may be at some site other than the nervous system; the changes in motor activity may be secondary, that is, a result of the primary effect.” Motor activity measurements alone may lack specificity, may not differentiate psychoactive and other chemicals from neurotoxicants, and are not likely to provide information about the origin of the activity change (Maurissen and Mattsson, 1989; Tilson, 1993). According to WHO (1986): “Activity is not a unitary measure and a change in the frequency of this behaviour can reflect toxicant-induced changes in one or more sensory or motor functions, alterations in reactivity (excitability) or motivational states, or perturbations of a variety of regulatory states (e.g., diurnal cycles, energy balance of the animal). For example, a decrease in activity might mean that the animal is paralysed or,

perhaps, that it suffers from 'general malaise'. In summary, a statistically significant difference in motor activity only is not a positive indicator of neurotoxicity.

The second component of our research entailed an investigation of neurotoxicity studies in which the only statistically significant effect was an increase in motor activity response. The goal was to determine how such studies were summarized and interpreted and whether the observed effect was determined to be biologically relevant or not. In conducting this research we discovered that keyword searching on "motor activity" or "locomotor activity" will identify thousands of undifferentiated toxicology studies. It is very difficult, however, to then narrow such broad search results to specifically identify only those studies in which the motor activity endpoint was the only statistically significant endpoint. We also encountered similar difficulties when we researched published literature for studies in which regulatory decision-makers evaluated stand-alone significant motor activity responses. Despite these difficulties, we were able to identify two studies that were responsive to our search, and these are discussed below.

One study evaluated the neurotoxicity potential of DEET using two measures: functional observational battery and motor activity measurements (Schoenig et al., 1993). Similar to the WIL (2003) study, the DEET analysis found statistically significant changes in motor activity, but concluded that the effects were "of minor or questionable biological significance based on the small magnitude of the changes and the transient nature of the findings." (Schoenig et al., 1993).

Ostergaard et al. (1993) evaluated the effects of white spirit inhalation in young and old rats. These rats were subjected to behavioral tests including motor activity and functional observational battery. In this study, white spirit inhalation caused an increase in motor activity in young rats albeit not a significant difference. No other changes were reported when a number of other neurotoxicity tests were conducted. The authors concluded that the techniques applied failed to reveal neurobehavioral white-spirit-induced CNS-neurotoxicity.

AMEC found in its literature search that changes in locomotor activity as a stand-alone endpoint in a neurotoxicity battery of tests is not considered by many toxicologists and regulatory agencies as a positive indicator of neurotoxicity. These conclusions result from the evaluation of science policy and regulatory guidance documents as well as two specific case studies culled

from the literature. Thus, AMEC's evaluation of the literature supports the conclusion made in the WIL (2003) report on resorcinol.

3.0 PART II: STATISTICAL ANALYSES

The following sections summarize the results of the additional statistical analyses performed first with the locomotor activity data and second with the follicular hyperplasia data.

3.1 LOCOMOTOR ACTIVITY

In the WIL (2003) report, evaluation of the locomotor activity in males on PND 61 showed a statistically significant increase for the 40 mg/L through 360 mg/L groups. This significance was determined first by subjecting the data to a parametric ANOVA to determine intergroup differences. If the ANOVA was significant ($p < 0.05$), Dunnett's test was used. This combination of statistical methods is typically employed in locomotor studies. AMEC evaluated this possible trend by using other methods, such as non-parametric statistics, as well as performing some additional exploratory data analyses. All statistical evaluations were performed using Minitab (v. 12.23; Minitab, 1999). The focus of this assessment is on the PND 61 male rats using data provided in Tables 92 and 193 from the WIL (2003) report.

AMEC first confirmed that the total and ambulatory data were normally distributed using the Shapiro-Wilk's test (r -values of 0.992 and 0.991, respectively). Results reported by WIL (2003) for the parametric ANOVA and Dunnett's test were verified using untransformed data. The results were then evaluated using the non-parametric equivalents - Kruskal-Wallis Test (for ANOVA) and Mann-Whitney U Test (for Dunnett's test). Our assessment showed that there was a significant difference across all of the dose groups (Kruskal-Wallis Test $p = 0.017$) and that the non-parametric pair-wise comparisons of control versus individual dose groups showed similar significant differences as were noted from the parametric tests (Table 3-1). The only exception was for the ambulatory results where the Mann-Whitney U Test showed a statistically significant difference in the comparison of the 40 mg/L dose relative to control ($p = 0.024$) while this was not observed using the parametric test.

The application of parametric and non-parametric tests to the PND61 male rat results suggests a potential positive relationship with dose. This was examined further by (1) performing the Mann-Whitney U Test on sequential pairs of results, and (2) performing regression analysis across the control and dose intervals. Table 3-2 summarizes these paired comparisons. Since none of the comparisons yielded p-values less than 0.05, it is unlikely that there is a positive relationship between locomotor effects and dose.

Graphing the individual results against the doses further supports the lack of a clear dose-effect relationship. Figures 3-1 and 3-2 show the data and fitted regression lines of the total and ambulatory data, respectively. In both cases the r^2 -values for the linear relationships are exceedingly poor, indicating that there is no clear dose-response relationship. Although non-zero slopes are calculated by the regression equation, the confidence bounds of the slopes are large (see figures), further supporting the lack of a strong and significant dose-response relationship. The r^2 -values are poor and large confidence bounds were calculated for the slopes, because of the high variability of the data and the significant overlap in the data across different dosing groups. The suggestion of a possible trend in the results from both the parametric and non-parametric statistical analyses are being “driven” by some elevated results in the higher dose groups.

Table 3-1. Summary of Parametric and Non-Parametric Statistical Test Results for Locomotor Activity Between Resorcinol Treatment Groups and Control (Male Rats Only)

Dose Group		Results		Parametric Test ¹		Non-Parametric Test ²	
		Total	Ambulatory	Total	Ambulatory	Total	Ambulatory
0 mg/L	Mean	1599	513	---	---	---	---
	SD	416	180				
10 mg/L	Mean	1990	624	NS ³	NS	NS	NS
	SD	606	221				
40 mg/L	Mean	2141	704	Sig (p < 0.05)	NS	Sig (p = 0.022)	Sig (p = 0.024)
	SD	590	217				
120 mg/L	Mean	2215	769	Sig (p < 0.05)	Sig (p < 0.01)	Sig (p = 0.003)	Sig (p = 0.006)
	SD	442	198				
360 mg/L	Mean	2254	787	Sig (p < 0.01)	Sig (p < 0.01)	Sig (p = 0.003)	Sig (p = 0.002)
	SD	521	222				

1. Based on Dunnett's Test as reported by WIL (2003)
2. Based on Mann-Whitney U Test
3. NS: Not statistically significant (alpha of 5%)

Table 3-2. Summary of Mann-Whitney U Test Results for Locomotor Activity Between Sequential Resorcinol Treatment Groups (Male Rats Only)

Comparison	Total	Ambulatory
Control v. 10 mg/L	NS (p = 0.085)	NS (p = 0.126)
10 mg/L v. 40 mg/L	NS (p = 0.592)	NS (p = 0.396)
40 mg/L v. 120 mg/L	NS (p = 0.768)	NS (p = 0.314)
120 mg/L v. 360 mg/L	NS (p = 0.942)	NS (p = 0.827)

Note:

NS: Not statistically significant (alpha of 5%)

Figure 3-1. Total Locomotion - Lack of Dose-Effect Relationship

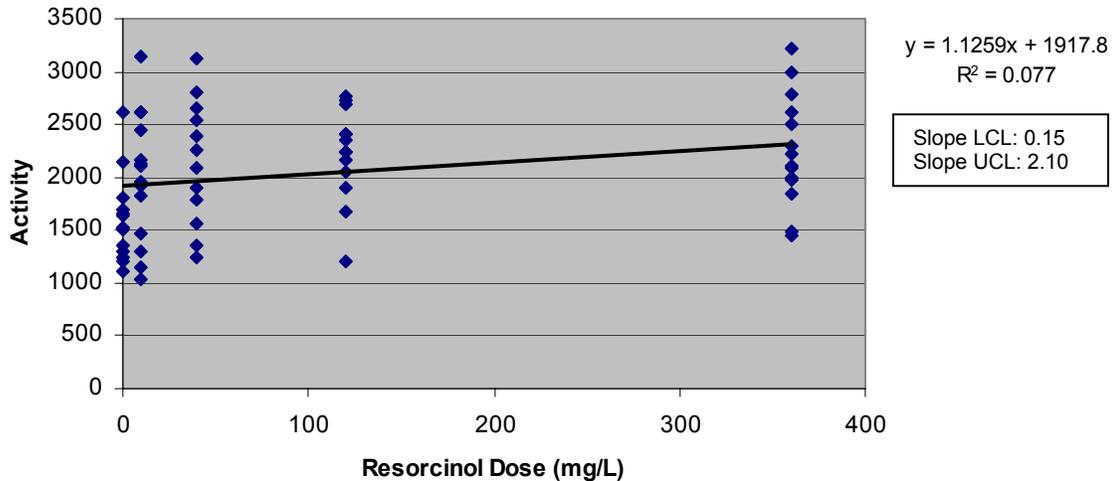
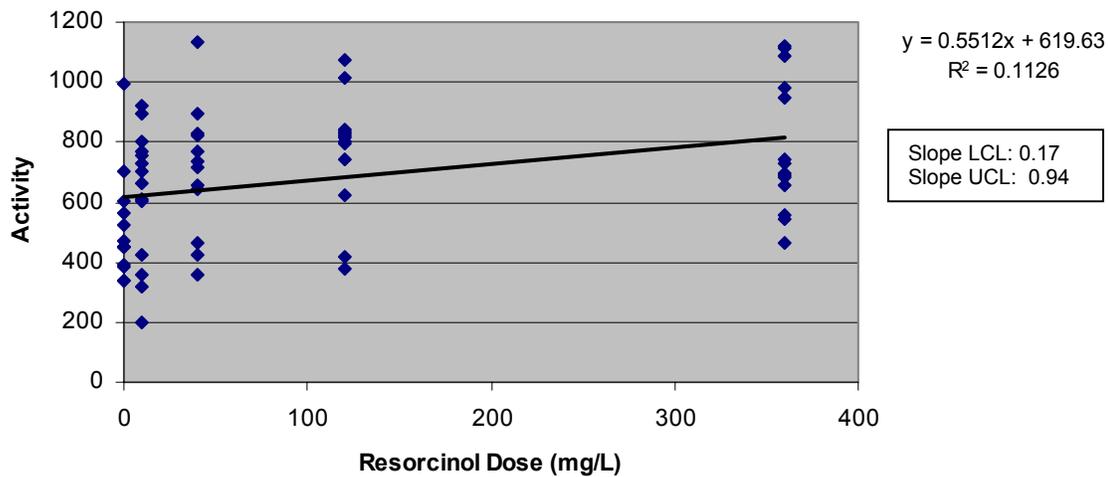


Figure 3-2. Ambulatory Locomotion - Lack of Dose-Effect Relationship



3.2 HYPERPLASIA INCIDENCE

In the WIL (2003)'s study, evaluation of the follicular hyperplasia data using Fisher's Exact Test showed no significant difference between treatments and control for male or female rats. The Fisher Exact Test calculates the exact probability of obtaining the result obtained by chance and is commonly used to assess quantal (all or none) data in a 2 x 2 contingency table format (Gad and Weil, 1988). Although AMEC confirmed the results from the WIL (2003) report, it was observed that the greatest frequency of occurrence was observed for males and females at the maximum (360 mg/L) dose. Consequently, alternative statistical tests were evaluated to determine whether or not the results of any dose groups were statistically significantly different from the control group and whether a trend in hyperplasia incidence with increasing dose might be present. All statistical evaluations were performed using Minitab (v. 12.23; Minitab, 1999), the PopTools Add-in to Excel (Hood, 2004), or OpenEpi (2004). The data used for this assessment were provided in Tables 50, 55, and 61 from the WIL (2003) report.

Initially, the 2x2 Chi-Square test was considered as an alternative to the Fisher's Exact Test but the former is not appropriate since (1) most cells in the 2x2 table had values less than 10, (2) more than 20% of the expected values were less than 5, and (3) the total sample size is less than 50 (Gad and Weil, 1988). Thus, other statistical tests were investigated. Table 3-3 summarizes the odds ratios, risk ratios, and their confidence bounds for the different combinations of control versus dose, and for the F0 male rats interim necropsy (identified as "Male, F0, short" in this table), F0 male rats scheduled necropsy (identified as "Male, F0, full" in this table), and for the F0 female rats scheduled necropsy (identified as "Female, F0, full" in this table). Odds ratios are appropriate for this data set because they are a simple way to compare whether the probability of a certain event is the same for two groups. An Odds Ratio of 1 means that there were no differences between the groups being compared. Odds Ratios can be greater than one or less than one, and the further away from 1 the Odds Ratio is, the stronger the potential effect. Review of this table suggests the potential for an effect at the higher dose ranges; however, in all cases the confidence limits overlapped 1.0 indicating that there were no significant differences between test groups and the control groups. Although these results support the conclusion from the Fisher's Exact Test, they were further evaluated with a larger dataset by combining the results across sexes and exposure durations (Table 3-4).

When both male groups were together (i.e., interim and scheduled F0 males), confidence limits of the Odds Ratios for this larger group still overlapped 1.0 for all dose groups. When all animals were grouped together (i.e., interim and scheduled F0 males and F0 females), confidence limits of the Odds Ratios for this larger group still overlapped 1.0, except for the comparison of controls and the 360 mg/L dose group. The latter result suggests a potential effect relative to control for the maximum dose group.

Table 3-3. Summary of Odds Ratios and Risk Ratios for Follicular Hyperplasia

Comparison	Males, F0, Short		Males, F0, Full		Females, F0, Full	
	Odds Ratio	Risk Ratio	Odds Ratio	Risk Ratio	Odds Ratio	Risk Ratio
Control v. 10 mg/L	0.22 (0.02-2.97)	0.33 (0.04-2.48)	0.22 (0.02-2.97)	0.33 (0.04-2.48)	0.56 (0.08-4.01)	0.62 (0.12-3.13)
Control v. 40 mg/L	1.0 (0.12-8.31)	1.0 (0.3-3.35)	2.67 (0.28-25.6)	1.56 (0.56-4.34)	0.67 (0.09-4.89)	0.72 (0.14-3.61)
Control v. 120 mg/L	3.33 (0.36-30.7)	1.67 (0.63-4.42)	1.0 (0.12-8.31)	1.0 (0.30-3.35)	0.28 (0.03-3.10)	0.33 (0.04-2.8)
Control v. 360 mg/L	8 (0.60-106.9)	2 (0.81-4.96)	3.33 (0.36-30.7)	1.67 (0.63-4.42)	3.33 (0.63-17.6)	2.17 (0.71-6.66)

Note:

Values shown in parentheses are the confidence limits.

Table 3-4. Summary of Odds Ratios and Risk Ratios for Follicular Hyperplasia Using Combined Datasets

Comparison	Males, F0, Full Plus Males, F0, Short		All Combined	
	Odds Ratio	Risk Ratio	Odds Ratio	Risk Ratio
Control v. 10 mg/L	0.22 (0.04-1.39)	0.33 (0.08-1.38)	0.33 (0.09-1.26)	0.43 (0.15-1.23)
Control v. 40 mg/L	1.55 (0.34-7.11)	1.26 (0.57-2.76)	1.13 (0.36-3.53)	1.08 (0.51-2.28)
Control v. 120 mg/L	1.78 (0.40-7.94)	1.33 (0.63-2.84)	1.0 (0.32-3.10)	1.0 (0.47-2.13)
Control v. 360 mg/L	4.89 (0.93-25.67)	1.83 (0.94-3.56)	3.6 (1.18-10.95)	1.93 (1.06-3.52)

Notes:

Values shown in parentheses are the confidence limits.

Potential trend tests for assessing these results were also evaluated. The Extended Mantel-Haenszel chi square test (OpenEpi, 2004) can be used to calculate the departure of a linear trend from horizontal (i.e., no trend). If the associated p value is less than 0.05, there is 95% probability that a trend exists in the underlying population. Application of this test to the F0-males-short and F0-females-full datasets indicated a potential trend in the data ($p = 0.014$ and 0.019 , respectively). AMEC has designated the results as "potential trends" because this test evaluates the odds ratios, which, as noted above, had large confidence intervals associated with them. The Jonckheere-Terpstra test is a commonly used non-parametric test to evaluate trends in data (Neuhauser et al., 1998). The two-sided p-values are summarized below:

- Males, F0, Short: $p = 0.0235$
- Males, F0, Full: $p = 0.1870$
- Females, F0, Full: $p = 0.1652$
- Males, F0, Full Plus Females, F0, Full: $p = 0.0538$
- All sexes and durations combined: $p = 0.0044$.

P-values greater than 0.05 (alpha of 5%) indicate no potential trend in the data. These results indicate a potential trend with dose for "Males, F0, Short" and "All sexes and durations combined".

4.0 CONCLUSION

Locomotor Activity Endpoint: AMEC's statistical analysis has concluded that there is no clear dose-response relationship in the locomotor test data. Of all the neurobehavioral tests executed, only the locomotor activity test was statistically significantly different from controls. Because there is no clear dose-dependence, because statistical significance was caused by one or two outlier animals, because this endpoint was the only neurobehavioral endpoint that was recorded as significantly different from controls, and because no effects on locomotor activity or other endpoints were seen in female animals or male animals of a younger age, AMEC agrees with WIL's conclusions that resorcinol in this study did not cause neurotoxic effects.

Thyroid Hyperplasia Endpoint: AMEC's statistical analysis of the hyperplasia results was inconclusive relative to identifying a LOAEL or a potential trend with dose when each animal

group was analyzed separately due to the large confidence bounds that were calculated using Odds Ratios and Risk Ratios because of the small sample sizes. However, both a LOAEL and a dose-dependent trend were indicated when animals were grouped to yield larger sample sizes. First, the 360 mg/L dose group was identified as a statistically significant LOAEL when all three groups were combined. Second, the application of the Jonckheere-Terpstra test demonstrated a statistically significant trend across doses when all groups were combined. Based on this analysis AMEC concludes that the 360 mg/L dose can be designated as a LOAEL for thyroid hyperplasia. However, use of the 360 mg/L dose as a LOAEL is very conservative. AMEC predicts that other toxicologists reviewing the same data may conclude that this dose is a NOAEL for thyrotoxicity.

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