Health Risk Assessment/Characterization of the Drinking Water Disinfection Byproducts Chlorine Dioxide & Chlorite

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FOREWORD

The purpose of this document is to provide scientific support and rationale for the hazard identification and dose-response information pertaining to chronic oral exposure to chlorine dioxide and chlorite. It is not intended to be a comprehensive treatise on the chemical or toxicology of chlorine dioxide or chlorite. Matters considered in this risk characterization include knowledge gaps, uncertainties, quality of data and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

An earlier draft of this document underwent external peer review by three independent experts and experts within EPA. The charge to external peer reviewers and their comments are presented in the Appendix. Reviewers' comments were considered in preparing the final version of this document.

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1.0 Introduction

Chlorine dioxide (ClO_2 ; CASRN 10049-04-4) is used as a drinking water disinfectant. Chlorine dioxide is a strong oxidizing agent, that under oxidant demand conditions, is readily reduced to chlorite (ClO_2-; CASRN 7758-19-2), another strong oxidizing agent. The Drinking Water Criteria Document on Chlorine Dioxide, Chlorite and Chlorate (U.S. EPA, 1994) provided the health effects data that support establishing derivation of a MCLG under Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986. This document updates and evaluates information from 1994 to 1997 relevant to the health risk assessment and characterization of the drinking water disinfectant, chlorine dioxide, and the degradation byproduct, chlorite. In particular, a two-generation reproduction study in rats conducted by the Chemical Manufacturers Association (CMA, 1996) is considered. The primary emphasis of this document is to integrate the noncancer oral toxicity data for key endpoints relevant to human health and to provide a hazard assessment/characterization, dose-response assessment/characterization, and risk characterization for chlorine dioxide and chlorite to support the Stage 1 Disinfectant/Disinfection By-Products (DBP) Rule.

Chlorine dioxide and chlorite are characterized together in this report since studies conducted with chlorite, the predominant degradation product of chlorine dioxide, are likely relevant to characterizing the toxicity of chlorine dioxide. In addition, studies conducted with chlorine dioxide may be relevant to characterizing the toxicity of chlorite. Chlorine dioxide is fairly unstable and rapidly dissociates predominantly into chlorite and chloride, and to a lesser extent, chlorate. There is a ready interconversion among these species in water (before administration to animals) and in the gut (after ingestion) (U.S. EPA, 1994). Therefore, what exists in water or the stomach is a mixture of these chemical species (i.e., chlorine dioxide, chlorite, chlorate) and possibly their reaction products with the gastrointestinal contents. As a result, the toxicity data for one compound are considered applicable for addressing toxicity data gaps for the other.

The key endpoints associated with chlorine dioxide and chlorite most relevant to human health effects are reproductive and neurodevelopmental toxicity (U.S. EPA, 1994). New data for these endpoints are reviewed in light of the new U.S. EPA guidelines for reproductive and developmental toxicity risk assessment (U.S. EPA, 1991, 1996a), and for neurotoxicity risk assessment (U.S. EPA, 1997a). An earlier draft of this document underwent peer review by three independent external experts and experts within EPA. Reviewers' comments (presented in the Appendix) were taken into consideration during preparation of this document.

2.0 Hazard Assessment/Characterization

In general, short-term exposure to chlorine dioxide and chlorite induces adverse hematological effects, including methemoglobinemia. These effects are described in detail in U.S. EPA (1994). Available studies in humans do not provide much insight into the health effects of long-term exposure to chlorine dioxide and chlorite. Ecological epidemiology studies are fraught with methodological problems that limit interpretation of the results. Animal studies consistently identify adverse effects on developmental and neurodevelopmental outcome. Carcinogenicity data from animal studies are largely inadequate to discern a carcinogenic hazard to humans.

2.1 Studies in Humans

Clinical reports of poisonings are not available for chlorine dioxide or chlorite (U.S. EPA, 1994). There are also no adequate long-term epidemiological studies to assess carcinogenicity of these chemicals (U.S. EPA, 1994; 1997c).

Short-term studies in humans reviewed in U.S. EPA (1994) include those by Lubbers et al. (1981, 1982, 1983) and Bianchine et al. (1981) wherein no alterations in hematological or urine chemistry or in physical symptoms were found in human volunteers administered up to 0.34 mg/kg chlorine dioxide in drinking water for one day or administered 0.036 mg/kg-day of chlorine dioxide or chlorite in drinking water for 84 days. In an epidemiological study of a community using chlorite as a drinking water disinfectant, adult exposures ranged 0.007-0.03 mg/kg-day for chlorine dioxide and 0.09-0.020 for chlorite for 12 weeks, and no consistent alterations in hematological parameters were reported (Michael et al., 1981). Tuthill et al. (1982) retrospectively compared morbidity and mortality data for a community that had utilized chlorite as a disinfectant with a neighboring community and found a greater postnatal weight loss in infants from the exposed community and no increase in the proportion of premature births when the age of the mother was controlled.

The study by Kanitz et al. (1996) is the only new epidemiologic study on chlorine dioxide or chlorite since the drinking water criteria document (U.S. EPA, 1994). Prompted by suggestions of disinfectant byproduct-induced adverse reproductive/developmental outcome from laboratory research and the results of Tuthill et al. (1982), Kanitz et al. (1996) conducted a cross-sectional study in Italy on the association between somatic parameters in infants at birth and drinking water disinfection with chlorine dioxide and/or sodium hypochlorite. This study, however, because of methodological problems, does not contribute new information as to whether there is an

association between adverse reproductive/developmental outcome in human populations and exposure to chlorine dioxide and/or chlorite in the drinking water.

During 1988–1989, Kanitz et al. (1996) followed 548 births at Galliera Hospital, Genoa, and 128 births at Chiavari Hospital, Chiavari, Italy. Data on infant birth weight, body length, cranial circumference and neonatal jaundice, and maternal age, smoking, alcohol consumption, education and pre-term delivery, were collected from hospital records. Women in Genoa were exposed to filtered water disinfected with chlorine dioxide, sodium hypochlorite or both; trihalomethane levels varied from 8 to 16 ppb in chlorite-treated water and 1 to 3 ppb in chlorine dioxide disinfected water. Women residing in Chiavari used water pumped from wells, without any disinfection treatment, and served as the comparison group (controls). Odds ratios were determined for the somatic parameters by comparison of groups exposed to chlorine dioxide, sodium hypochlorite, or both, with controls, and adjusted for maternal education level, income, age, smoking and sex of the child. Neonatal jaundice occurred more frequently (odds ratio, OR = 1.7; 95% confidence interval, CI = 1.1–3.1) in infants whose mothers resided in the area where surface water was disinfected with chlorine dioxide, when compared to infants with mothers using nondisinfected well water. Infants born to mothers residing in areas where surface water was disinfected had smaller cranial circumference (≤ 35 cm; OR= 2.2; 95% CI= 1.4-3.9 for chlorine dioxide; OR = 3.5; 95% CI = 2.1–8.5 for sodium hypochlorite vs. untreated well water; OR = 2.4; 95% CI = 1.6–5.3 for both vs. untreated well water). In addition, these infants had a smaller body length (≤ 49.5 cm; OR = 2.0; 95% CI = 1.2–3.3 for chlorine dioxide vs. untreated well water; OR = 2.3; 95% CI = 1.3–4.2 for chlorite vs. treated well water). Risks for low-birth-weight infants (≤ 2500 g) were reported to be increased in mothers residing in areas using water disinfected with chlorite and chlorine dioxide, but these associations were not statistically significant. For pre-term delivery (≤ 37 weeks), small but not statistically significant increased risks were found among mothers residing in the area using chlorine dioxide. The authors concluded that infants of women who consumed drinking water treated with chlorine compounds during pregnancy were at higher risk for neonatal jaundice, cranial circumference ≤ 35 cm and body length ≤ 49.5 cm.

The interpretability of the results of Kanitz et al. (1996) is limited by lack of consideration of exposure and potential confounding variables, such as the quantity of water consumed during pregnancy, lack of quantitative exposure information, exposure to other chemicals in the water, and nutritional habits, amount of smoking and age distribution of the women. In addition, baseline values for the infant sex ratio and percentage of low-weight births for the comparison group deviate from values presented by the World Health Organization for Italy. For example, the sex ratio used in the study for the comparison group was 86, but most recent data (1991) for Italy indicate a sex ratio

value of 112. While the percentage of low-weight births in the control group for the Kanitz et al. (1996) study was 0.8%, in Italy for 1992. the percentage of low-weight births (< 2,500 grams) is 6.7%. The quality of the untreated well water is not known, i.e., whether it contained any chemical or biological contaminants. The atypical baseline data raise concerns about the control population selected for this study and render any comparison to them by the exposed group difficult to interpret, thereby precluding the ability to draw conclusions (Selevan, 1997).

Short-term drinking water studies with chlorine dioxide or chlorite in human volunteers and ecological studies in communities using these chemicals for disinfection of water supplies suggest that additional research into potential adverse effects, including hematologic, reproductive and developmental outcome in human populations is needed. The studies thus far do not form an adequate basis to conclude that exposure to chlorine dioxide and/or chlorite in drinking water results in adverse reproductive/developmental health effects in humans (U.S. EPA, 1997b). Methodological problems, such as control for confounding variables, identification of appropriate control or comparison groups, quantification of exposure, and simultaneous exposure to multiple chemicals, are among the factors that limit the interpretation of the results.

2.2 Studies in Animals

2.2.1 Toxicokinetics

It is difficult to obtain data on the toxicokinetics of chlorine dioxide and chlorite because of their chemical reactivity. However, a series of experiments in rats by Abdel-Rahman and coworkers (Abdel-Rahman , 1985; Abdel-Rahman et al., 1980a,b; 1982; 1984a; Suh and Abdel-Rahman, 1983; reviewed in U.S. EPA, 1994) using radioactive chlorine as a tracer suggest that following oral exposure, chlorine dioxide and chlorite are rapidly absorbed (approximately 35-40% absorbed), widely distributed in the body, and are rapidly and predominantly excreted in the urine in the form of chloride (Cl-). Chlorine dioxide appears to be altered after coming into contact with organic material in the gastrointestinal tract and may also be reduced by saliva. It is not clear whether it is the parent compounds or degradation products that are actually absorbed. There is no evidence that these chemicals bioaccumulate.

2.2.2 Noncancer Effects

2.2.2.1 Systemic Toxicity

U.S. EPA (1994) discusses at length the effects observed in chlorine dioxide and chlorite studies utilizing various exposure durations. Tables 1 and 2 summarize the subchronic and chronic toxicity information presented in U.S. EPA (1994); the reader is directed to the criteria document for detailed information about these studies. In general, the systemic toxicity studies presented in the criteria document are of limited quality for risk assessment.

Groups of Sprague-Dawley rats (10/sex) were administered chlorine dioxide in drinking water for 90 days at concentrations of 0, 25, 50, 100 or 200 mg/L (Daniel et al., 1990). These concentrations correspond to doses of 0, 2, 4, 6 or 12 and 0, 2, 5, 8 and 15 mg/kg-day ClO_2 for males and females, respectively (conversion provided by authors). A significant increase in the incidence of nasal lesions (goblet cell hyperplasia and inflammation of nasal turbinates) was found in the exposed animals. However, the authors postulated that these lesions were most likely due to inhalation of ClO_2 vapors at the drinking water sipper tube or from off-gassing of the vapors after drinking rather than ingestion of the drinking water. Thus, the 2 mg/kg-day dose group could be described as a LOAEL, but the toxicological significance of the findings was inconclusive since the lesions were not reported in any other studies and may possibly be an artifact of treatment.

Revis et al. (1986) evaluated the effects of chlorine dioxide and chlorite on male pigeons. Pigeons were exposed to water concentrations of either 0, 0.07, or 0.5 mg/kg-day chlorine dioxide or chlorite for three months. The birds were maintained on either a low calcium or low calcium/high lipid diet; no exposed animals were maintained on a normal diet. Increased serum cholesterol and decreased thyroid hormone levels were observed in some groups, but there was no dose-response effect. Statistically significant increases in aortic plaque size were observed in the 0.07 mg/kg-day group fed the low-calcium diet. Therefore, the 0.07 mg/kg-day dose could be described as a LOAEL; however, the relevance of the findings to animals on a normal diet is unclear. In order to answer this question of relevance, Penn et al. (1990) repeated the study using chlorite doses of 0, 0.26, and 2 mg ClO_2 mg/kg-day, maintaining the pigeons on a low calcium diet. There was no significant difference in any of the parameters measured that could be attributed to the ingestion of chlorite. Therefore, the 2 mg/kg-day dose is considered a NOAEL.

Table 1. Summary of Subchronic and Chronic Toxicity Studies for Chlorine Dioxide						
Reference	Species, Sex	Route, Doses (mg/kg-d)	Duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Endpoint
Daniel et al. (1990)	Rat, M	water, 0, 2, 4, 6, 12	90 days	—	2	nasal goblet cell hyperplasia, inflammation of nasal turbinates; concern that results not related to ingestion of chlorine dioxide
	F	0, 2, 5, 8, 15		—	2	same as males and reduced spleen weight
Revis et al. (1986)	Pigeon, M	water, 0, 0.07, 0.5	3 months	—	0.07	elevated cholesterol, decreased thyroid hormones; not dose-dependent increased aortic plaque size all treated groups were fed either low calcium or low calcium-high lipid diet; relevance of findings to animals with normal diet not clear
Abdel-Rahman et al. (1980a); Couri and Abdel-Rahman (1980); Abdel-Rahman et al. (1984b)	Rat, M	water, 0, 0.1, 1, 10	up to 1 year	ND	ND	hematological effects (including increased catalase activity, increased resistance of RBC to hemolysis); effects not dose-related; unclear if effects are either statistically or biologically significant
	Mice, M	water, 0, 0.18, 1.8, 18				
Haag (1949)	Rat, M, F	water, 0, 0.07, 0.13, 0.7, 1.3, 13	2 years	1.3	13 (FEL)	decreased survival; study limited by insufficient numbers of animals and inadequate evaluation of sensitive parameters

FEL= frank effect level
ND= not determined

Table 2. Summary of Subchronic and Chronic Toxicity Studies for Chlorite						
Reference	Species, Sex	Route, Doses (ClO_2-) (mg/kg-d)	Duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Endpoint
Revis et al. (1986)	Pigeon, M	water, 0, 0.07, 0.5	3 months	0.5	---	elevated cholesterol and LDL; not dose-dependent increased aortic plaque size; not dose-dependent all treated groups were fed either low calcium or high lipid diet; relevance of findings to animals with normal diet not clear
Penn et al. (1990)	Pigeon, M	water, 0, 0.26, 2	3 months	2	---	no effects on cholesterol and aortic plaque size; repeat of the Revis et al. (1986) study- animals maintained on low calcium diet
Couri and Abdel-Rahman (1980); Abdel-Rahman et al. (1984b)	Rat, M	water, 0, 1, 10	6-12 months	ND	ND	decreased GSH, decreased osmotic fragility; effects not dose related; unclear if effects either statistically or biologically significant
Haag (1949)	Rat, M, F	water, 0, 0.09, 0.18, 0.35, 0.7, 9.3, 81	2 years	0.7	9.3	renal effects; effects attributed to a nonspecific salt effect by authors
Ridgway (1992); Harrington et al. (1995a)	Rat, M, F	gavage, 0, 8, 19, 60	13 weeks	7.4	18	increased spleen and adrenal weights; histopathologic changes in gastric mucosa

ND = not determined

A series of studies was conducted to examine the effects of drinking water exposure to chlorine dioxide and chlorite on hematological parameters (Abdel-Rahman et al., 1980a: Couri and Abdel-Rahman (1980), Abdel-Rahman et al., 1984b). Male rats were exposed for up to one year to chlorine dioxide doses of 0, 0.1, 1, or 10 mg/kg-day, or to chlorite doses of 0, 1 or 10 mg/kg-day. A number of hematological parameters were measured including red blood cell counts, osmotic fragility, hematocrit, and levels of the following: hemoglobin, glutathione reductase, glutathione peroxidase, catalase, glutathione and methemoglobin. Some alterations in most parameters were observed. For chlorine dioxide, the effects included increased catalase activity and increased resistance of red blood cell (RBC) to hemolysis. For chlorite, the observed effects include decreased glutathione (GSH) and decreased osmotic fragility. However, there was no consistent relationship with dose and effects were not observed consistently throughout the period of exposure. Also, it was unclear if any of the effects were statistically or biologically significant. Therefore, this series of studies was insufficient for determining a NOAEL or LOAEL.

Haag (1949) conducted the only chronic study that evaluated the effects of chlorine dioxide or chlorite in drinking water. Chlorine dioxide at concentrations of 0, 0.5, 1, 5, 10 or 100 mg/L (0, 0.07, 0.13, 0.7, 1.3 or 13 mg/kg-day) or chlorite at concentrations of 0, 1, 2, 4, 8, 100 or 1000 mg/L (0, 0.09, 0.18, 0.35, 0.7, 9.3 or 81 mg/kg-day) was administered to rats (7/sex/ group) for two years. For chlorine dioxide, the survival of animals consuming water containing 10 mg ClO_2/L (1.3 mg/kg-day) or below was not significantly affected; survival in the 100 mg/L group was significantly decreased. Histopathologic studies were also performed on representative animals from each dose group, but no correlation was observed between treatment and any pathological finding. This study identified a NOAEL of 1.3 mg ClO_2/kg-day and a FEL (based on decreased survival) of 13 mg ClO_2/kg-day. Animals exposed to chlorite concentrations of 100 or 1,000 mg/L (9.3 or 81 mg ClO_2-/kg-day) exhibited treatment-related renal pathology, characterized by distention of the glomerular capsule and appearance of a pale pinkish staining material in the renal tubules. These effects were also observed in a group of animals that had been administered sodium chloride at a concentration equimolar to 1,000 mg $NaClO_2$/L. The author concluded that renal pathology was a nonspecific salt effect, but this observation does not alter the observation that concentrations of 100 mg/L or higher lead to adverse effects. Based on renal effects, this study identified a NOAEL of 0.7 mg ClO_2-/kg-day and a LOAEL of 9.3 mg ClO_2-/kg-day. The study was limited because there was an insufficient number of animals tested per group, pathology was conducted on a small number of animals, and it did not provide adequate evaluations of more sensitive parameters, which would have been more useful in the overall assessment of chronic toxicity.

One subchronic toxicity study of sodium chlorite in rats, a 13-week gavage study (Harrington et al.. 1995a), has been published since the drinking water criteria document (U.S. EPA, 1994). This study was previously described in U.S. EPA (1994) as Ridgway (1992), based on laboratory report of the study.

Harrington et al. (1995a), treated Crl:CD (SD) BR rats (15/sex/group) with sodium chlorite via daily gavage for 13 weeks at dose levels of 0, 10, 25, or 80 mg/kg-day (equivalent to 0, 7.4, 18.6, or 59.7 mg ClO_2^-/kg-day, respectively), followed by terminal sacrifice. In the 59.7 mg/kg-day group, four animals died during treatment and both sexes exhibited salivation, significantly decreased RBC counts, and decreased total serum protein levels. The males receiving 59.7 mg/kg-day exhibited significantly decreased hematocrit and hemoglobin levels and increased methemoglobin and neutrophil levels, while in the females, methemoglobin levels were significantly decreased. Possible reasons for the decrease in methemoglobin in females, which is unexpected considering the known oxidative effects of sodium chlorite, was not discussed by the authors. Morphological changes in erythrocytes in some animals of both sexes and significant increases in relative adrenal and spleen weights in the males and absolute and relative spleen and adrenal weight, and relative liver and kidney weights in the females were also noted at 59.7 mg/kg-day. Body weight and food consumption were not affected by treatment. Histopathologic alterations in the 59.7 mg/kg-day group included squamous epithelial hyperplasia, hyperkeratosis, ulceration, chronic inflammation, and edema in the stomach of seven males and eight females. At 18.6 mg/kg-day, the following alterations were reported: occasional salivation in two males, hematological alterations (increased methemoglobin levels and neutrophil count, decreased lymphocyte count) in males, increases in absolute and relative spleen and adrenal weights in the females, and histologic alterations in the stomach of two males, similar to that seen in the high-dose group. The increase in absolute splenic weight was attributed to morphological alterations in erythrocytes but no explanation was provided for alterations in absolute adrenal weight. The NOAEL in this study was determined to be 7.4 mg/kg-day, and the LOAEL is 18.6 mg/kg-day for rats subchronically treated with sodium chlorite.

In conclusion, no subchronic or chronic studies adequately assess the systemic toxicity of chlorine dioxide. The study by Haag (1949) identifies a NOAEL of 1.3 and a LOAEL of 13 mg ClO_2/kg-day. However, the adequacy of this study is questionable. For chlorite, one adequate subchronic study (Harrington et al., 1995a) identified a NOAEL of 7.4 and a LOAEL of 18.6 mg ClO_2^-/kg-day, respectively, based on increases in absolute and relative spleen and adrenal weights and histopathologic changes in the gastric mucosa.

2.2.2.2 Reproductive and Developmental Studies

Discussed at length in the criteria document (U.S. EPA, 1994), are numerous reproductive and developmental studies for chlorine dioxide and chlorite. Rats exposed to chlorine dioxide prior to mating and throughout gestation (Carlton et al., 1991; Mobley et al., 1990; Orme et al., 1985; Suh et al., 1983; Taylor and Pfohl, 1985) produced pups with alterations in brain development (decreased brain weight, decreased brain cell number), thyroxine levels, and behavior (decreased exploratory behavior, locomotor activity). Similar studies with chlorite found either no significant treatment-related effects on rat development (Suh et al., 1983), or decreased thyroxine levels (Carlton and Smith, 1985), decreased pup weight and growth (Moore and Calabrese, 1982), or decreased exploratory behavior in pups (Mobley et al., 1990).

Studies looking at reproductive endpoints in males showed decreased testicular DNA synthesis (Suh et al., 1984; Abdel-Rahman et al., 1984b) and abnormal sperm morphology (Meier et al., 1985) for both chemicals, and decreased sperm motility with chlorite (Carlton and Smith, 1985; Carlton et al., 1987).

Subsequent to the 1994 criteria document, the U.S. EPA re-evaluated the Mobley et al. (1990) study in light of the weight of evidence of the newer studies. Two reproductive/ developmental studies with sodium chlorite in drinking water have recently been published. Harrington et al. (1995b) conducted a teratology study in female rabbits. This study was previously described in U.S. EPA (1994) as Irvine (1990), based on the laboratory report of the study. Chemical Manufacturers Association conducted a 2-generation study in rats that also included a neurotoxicity assessment (CMA, 1996). These reproductive and developmental studies for chlorine dioxide and chlorite are summarized in Tables 3 and 4, respectively.

Mobley et al. (1990) exposed female rats for 9 weeks to drinking water containing 0, 20 or 40 ppm sodium chlorite (0, 3 or 6 mg ClO_2^-/kg-day) beginning 10 days prior to breeding with untreated males and until the pups were sacrificed at 35-42 days postconception (PCD). Slight delays in exploratory behavior on PCD 36-37 were observed in rat pups exposed to 3 mg/kg-day. Animals exposed to 6 mg/kg-day exhibited a more consistent and significant depression in exploratory behavior occurring on PCD 36-39. Exploratory activity was comparable between the treated and control groups after PCD 39. Reviewing the results of this study relative to the findings of the newer developmental studies in the database, suggests the NOAEL for neurodevelopmental behavior effects in rats for this study is approximately 3 mg/kg-day and the LOAEL is 6 mg/kg-day.

Harrington et al. (1995b) treated groups of 16 female New Zealand white rabbits with technical grade sodium chlorite (80.6% purity) via their drinking water at levels of 0, 200, 600 or 1200 ppm from days 7 to 20 of pregnancy (gestational days, GD 7-20), followed by terminal sacrifice at day 28. Water concentrations were maintained at the same levels throughout pregnancy and were not adjusted for changes in volume of water consumed. Based on measured water consumption, the authors calculated a mean daily intake of approximately 0, 10, 26 or 40 mg ClO_2-/kg-day (corrected for purity and adjusted by the weight of the salt). Clinical condition, maternal body weight, and food and water consumption were measured daily. At necropsy, gravid uterine weights, number of corpora lutea, number of implantation sites, and live and dead fetuses were recorded. Live fetuses were weighed, examined for external abnormalities, sexed, dissected and a gross visceral examination performed. Skeletal examinations were also performed. Abnormalities were categorized as minor or major, the latter were thought to impair survival or fitness; commonly observed variations were also recorded. The authors did not state which malformations fell into each of these categorizes. There was no mortality, although two rabbits (one from each the control and 26 mg/kg-day groups) were sacrificed *in extremis* due to a clinical condition unrelated to treatment. A significant decrease in water consumption during the treatment period was observed in the 26 and 40 mg/kg-day groups, and a decrease during treatment days 16-20 of pregnancy was observed in the 10 mg/kg-day group. The authors attributed the decreases in consumption to lack of palatability of the drinking water solution, although no supporting data were presented. Food consumption was decreased in the 26 and 40 mg/kg-day groups during days 7-11 of pregnancy. Body weight gain of treated animals was decreased on days 7-19, although by day 26 these groups showed no differences from controls in body weight gain. The authors concluded there were no treatment-related effects on pregnancy incidence, number of implantations, number of preimplantation losses, fetal sex ratio, number of live fetuses or fetal visceral or structural abnormalities. Data for specific malformations and variations were not shown, instead data were presented as the number or mean percentage of fetuses with major or minor external and visceral or skeletal abnormalities. The number and mean percentage of major external and visceral, and skeletal abnormalities were increased in the 26 and 40 mg/kg-day groups (external/visceral: 6.6 and 2.9%, respectively, vs. 1.5% in controls; skeletal: 5.4 and 0%, respectively, vs. 0% in controls). Mean fetal weights in the 26 and 40 mg/kg-day groups were slightly decreased (<9% relative to controls). In the 26 and 40 mg/kg-day groups, the incidence of minor skeletal abnormalities (13.9 and 14.2 for the 26 and 40 mg/kg-day groups, respectively, vs. 7.7 % in controls) and skeletal variants related to incomplete fetal bone ossification (such as of the pubis and sternebrae) is higher than controls. The authors state in their discussion that these alterations in fetal body weight.

Table 3. Summary of Reproductive and Developmental Studies for Chlorine Dioxide

Reference	Species, Sex	Route, Doses (mg/kg-d)	Duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Endpoint
Suh et al. (1983)	Rat, F	water, 0, 0.1, 1, 10	2.5 mos, then gestation	1	10	decreased numbers of implants & live births
Orme et al. (1985)	Rat, F	water, 0, 1, 3, 14	2 wks prior mating through lactation	3	14	decreased exploratory & locomotor activity, decreased T3 & T4 in pups
	Rat, M	gavage, 0, 14	PND 5-20	—	14	delayed development, decreased T3 & T4, decreased exploratory & locomotor activity
Taylor & Pfohl (1985)	Rat, F	water, 0, 14	2 wks prior mating through lactation	—	14	decreased brain weight & cell number, decreased exploratory behavior in pups
	Rat, M	gavage, 0, 14	PND 5-20	—	14	decreased brain cell number & running wheel activity
Toth et al. (1990)	Rat, pups	gavage, 0, 14	PND 1-20	—	14	decreased forebrain weight & protein content
Mobley et al. (1990)	Rat, F	water, 0, 14	10 d prior mating, gestation, lactation to PCD 35-42	—	14	decreased litter weight & exploratory activity (PCD 36-39)
Carlton et al. (1991)	Rat, M/F	gavage, 0, 2.5, 5, 10	M: 56 d prior mating F: 2 wk prior mating through lactation (73 d total)	10	—	no effect on reproductive parameters or thyroid hormone levels in pups or adults
Suh et al. (1984)	Rat, M	water, 0, 1, 10	3 wks	1	10	decreased testicular DNA synthesis; biological significance unclear
Abdel-Rahman et al. (1984b)	Rat, M	water, 0, 1, 10	3 months	—	1	decreased testicular DNA thymidine incorporation; biological significance unclear
Meier et al. (1985)	Mice, M	gavage, 0, 3.2, 8, 16	5 d	16	—	no sperm abnormalities 1, 3, 5 wks after dosing

PCD= post conception day
PND= post natal day

Table 4. Summary of Reproductive and Developmental Studies for Chlorite						
Reference	Species, Sex	Route, Doses (ClO_2-) (mg/kg-d)	Duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Endpoint
Moore & Calabrese (1982)	Mice, F	water, 0, 22	mating through weaning	—	22	decreased pup weight & growth rate
Suh et al. (1983)	Rat, F	water, 0, 0.1, 1	2.5 mos prior to breeding then gestation	1	—	no skeletal & soft tissue fetal anomalies
Mobley et al. (1990)	Rat, F	water, 0, 3, 6	10 d prior mating, gestation, lactation to PCD 35-42	3	6	decreased exploratory activity (PCD 36-39); increase free T4 in pups
Irvine (1990); Harrington et al. (1995b)	Rabbit, F	water, 0, 10, 26, 40	gestation days 7-20	10	26	decreased fetal weight & delayed ossification; decreased body weight gain & food & water consumption in dams
CMA (1996)	Rat, M/F	water, 0, 2.9-3.8, 5.6-7.9, 20-28.6	2- generation study- premating, gestation, lactation	2.9	5.9	decreased absolute brain weight F1 & F2; lowered auditory start in F1 & F2; altered liver weights in F0 & F1
Carlton & Smith (1985); Carlton et al. (1987)	Rat, F	water, 0, 0.075, 0.75, 7.5	14 d prior mating through lactation	0.75	7.5	decreased T3 & T4 in pups
	M	water, 0, 0.075, 0.75, 7.5, 27	72-76 d	0.75	7.5	decreased sperm motility & increased abnormal morphology
Suh et al. (1984)	Rat, M	water, 0, 0.1, 1	3 wks	0.1	1	alterations in testicular & liver DNA synthesis; biological significance unclear
Abdel-Rahman et al. (1984b)	Rat, M	water, 0, 1, 10	3 months	—	1	decreased testicular DNA thymidine incorporation ; biological significance unclear
Meier et al. (1985)	Mice, M	gavage, 0, 8, 20, 40	5 d	40	—	no sperm abnormalities 1, 3, 5 wks after dosing

ND= not determined; PCD= post conception day; PND= post natal day

and delayed ossification are an indication of embryonic growth retardation. The NOAEL for this study is 10 mg ClO_2-/kg-day and the LOAEL is 26 mg/kg-day based on decreased fetal weight and delayed skeletal ossification, and decreases in food and water consumption, and decreased body weight gain in the dams.

While this study employed sufficient numbers of animals and administered chlorite by a route relevant to human exposure, uncertainties exist in the interpretation of the results due to inadequate reporting of the number and types of specific abnormalities and variations. There is additional uncertainty as to whether the decreases in food and water consumption and body weight gain in the dams are due to unpalatability or a direct toxic effect of the chlorite.

CMA (1996) conducted a two-generation study to better define the NOAEL and LOAEL for reproductive, developmental neurotoxicity and hematological endpoints. Thirty males and 30 female Sprague-Dawley rats of the OFA(SD)IOPS-Caw strain (F0) generation received drinking water containing 35, 70 or 300 ppm sodium chlorite (concentrations of sodium chlorite in the drinking water were apparently adjusted to compensate for the 81.4% purity of the test material) for 10 weeks and were then paired (1M:1F) for mating. A similar group received purified water and served as controls. Males were exposed throughout mating and then were sacrificed. Exposure for the females continued through mating, pregnancy, lactation and until necropsy following weaning of their litters. Sodium chlorite concentrations were adjusted downward during lactation to offset increases in the volume of water consumed so that a constant intake (mg/kg-day) could be maintained. Twenty-five males and females from each of the first 25 litters to be weaned in a treatment group were chosen to produce the F1 generation. The F1 pups were continued on the same treatment regimen as their parents. At approximately 14 weeks of age, they were mated to produce the F2a generation. Due to a reduced number of litters in the 70 ppm F1-F2a generation, the F1 animals were remated following weaning of the F2a to produce the F2b generation. Pregnant F1 females were allowed to litter and rear the F2a and F2b generations until weaning at postnatal day (PND) 21. Based on water consumption measured by the authors, and adjusting for the molecular weight of sodium in sodium chlorite, EPA-calculated doses for the F0 animals were 0, 3.0, 5.6, 20.0 and 0, 3.8, 7.5, and 28.6 mg ClO_2-/kg-day for males and females, respectively. For the F1 animals, doses were 0, 2.9, 5.9, and 22.7 mg ClO_2-/kg-day for the males and 0, 3.8, 7.9, and 28.6 mg ClO_2-/kg-day for the females. Numerous parameters were measured or calculated, including the following: body weight, food consumption, water consumption, estrus cycle in the F0 and F1, hematology and triidothyroxine and thyroxine levels in the F1 (blood samples collected from one male and one female from the first 20 F1 litters at age PND 25 and another group at 13 weeks),

gestation duration, litter size, pup sex, pup body weight, pup developmental landmarks, number alive/dead pups in the F1 and F2 generation, total caudal sperm number and percent motile, and morphology by computer assisted sperm motility analysis in the F0 and F1, and organ weight and histopathological examination of the brain, pituitary gland, liver, adrenal, spleen, thymus, kidneys, and reproductive organs of all F0 and F1 controls and high-dose animals. An additional group of F1 pups was chosen for neurohistopathology on PND 11 (examination of the brain and spinal cord) or PND 60 (sensory ganglia, dorsal and ventral nerve roots, and several peripheral nerves and muscles). Another group of F1 rats were examined for neurotoxicological endpoints (motor activity in a Figure 8 Activity System and neuropathology on PND 60, auditory startle in the SR-Screening System, learning and memory retention in a water E-maze). A functional observational battery (FOB) was also conducted on the pups undergoing the auditory and learning assessments. This group was composed of 2 males and 2 females from twenty litters and exposure was discontinued after weaning. A re-evaluation of the auditory startle response was conducted in 20 males and 20 females in the F2a and F2b generations.

There were reductions in water consumption, food consumption and in body weight gain in both sexes in all generations at various times throughout the experiment (e.g., during premating, pregnancy, gestation, post-weaning), primarily in the 70 and 300 ppm groups. The authors attributed these reductions to a lack of palatability of the drinking water solution, but did not show data to support this contention. Significant alterations related to treatment at 300 ppm include: reductions in absolute and relative liver weight in F0 females and F1 males and females, reduced pup survival (increase in number of pups found dead and/or killed prematurely during lactation) and reduced body weight at birth and throughout lactation in F1 and F2, lower thymus and spleen weight in both generations, lowered incidence of pups exhibiting a normal righting reflex and with eyes open on PND 15, alteration in clinical condition in F2 animals chosen for neurotoxicity, decreases in absolute brain weight for F1 males and F2 females, delays in sexual development in males (preputial separation) and females (vaginal opening) in F1 and F2, and lower red blood cell parameters in F1. It is possible that the reported alterations in pup sexual maturation measures may be due to reduced pup body weight, but a definitive conclusion cannot be drawn. In the 70 ppm groups, reduced absolute and relative liver weight in F0 females and F1 males and females, reduced absolute brain weight in F1 and F2, and minor changes in red blood cell parameters in F1. In addition, a significant decrease in maximum response to an auditory startle stimulus, was noted in the 70 and 300 ppm groups on PND 24, but not on PND 60. Analysis of the E-maze data by EPA personnel indicate possible alterations in learning behavior in the 70 ppm group, but the differences from the conclusions of the report could not be resolved. Minor changes in red blood cell parameters were apparent in the F1 at 35 ppm.

This is an adequate study since it was conducted with sufficient numbers of animals of both sexes and examined numerous endpoints. The study is acceptable and consistent with the U.S. EPA testing guidelines that were in effect at the time of the study (U.S. EPA, 1992). However, there are several limitations with the study. Lack of pair-watered and fed control animals confounds the results and precludes making definitive conclusions as to whether the alterations in food and water consumption and body weight are related to water palatability or a direct toxic effect of the agent. Developmental landmarks (e.g., vaginal opening in F2a group) were not reported for all groups. Grip strength and landing foot splay were not included in the functional observational battery (FOB). Discontinuation of exposure for the animals undergoing neurotoxicity testing minimizes the likelihood of finding a positive effect and precludes comparison of the data with that of other rats with continued exposure. While the study employed an exposure regimen consistent with testing guidelines and should potentially detect adverse effects on the developing nervous system, discontinuation of exposure after weaning reduces the opportunity to detect neurological effects from continuous or lifetime exposures similar to those expected from lifetime drinking water exposure in humans.

Interpretation of the neurobehavioral tests are limited. The report lacks detailed descriptions of experimental methods (e.g., size of the arena, length of observations) and positive control data (including estimates of variability) for the FOB. Positive control studies for the motor activity and E-maze studies used high doses of the validation chemicals and were not adequate to show the sensitivity of the methods, and showed only that effects of the chemicals at maximally toxic doses could be recognized. Variability in the startle response data was high. The high variability and problems in calibrating and operating the automated startle apparatus (as presented in the report) would tend to decrease the sensitivity of the test to detect a difference between control and treated groups, since differences in startle amplitude would have to be larger to attain statistical significance. In some cases, inappropriate statistical analysis were applied. For example, repeated-measures techniques were apparently not used to account for the fact that the rats were tested repeatedly, and it is not clear how non-parametric rank data were analyzed or why a log transformation was applied to the learning data.

Minor, statistically significant changes in hematological data at the 35 and 70 ppm concentrations (generally 1-7%) appear to be within normal ranges based on historical data and are, therefore, not considered clinically or biologically significant or adverse. The NOAEL for these hematological effects is considered to be 70 ppm.

The NOAEL for this study is 35 ppm (2.9 mg ClO_2-/kg-day) and the LOAEL is 70 ppm (5.9 mg ClO_2-/kg-day) based on lowered auditory startle amplitude, decreased absolute brain weight, and altered liver weights in two generations.

2.2.3. Carcinogenicity

There have been no long term oral bioassays for the carcinogenicity of chlorine dioxide. As reviewed by U.S. EPA (1994), short-term assays with oral administration of concentrates from drinking water treated with chlorine dioxide did not induce lung adenomas in Strain A mice, did not increase skin tumor frequency in an initiation-promotion assay, nor increase gamma glutamyl transpeptidase-positive foci in rat liver slices (Miller et al., 1986). Robinson et al. (1986) demonstrated induction of hyperplastic responses in the epidermis of SENCAR mice following 10-minute immersion in >300 mg ClO_2/L solutions.

Long-term studies in rats and mice do not provide sufficient evidence to draw conclusions as to the carcinogenic potential of chlorite in humans. No increase in tumor incidence relative to concurrent controls was found in male or female F344 rats drinking 0, 300 or 600 ppm (0, 18 or 32 and 0, 28 or 41 mg ClO_2-/kg-day in males and females, respectively) sodium chlorite for 85 weeks (Kurokawa et al., 1986). In another study, when Kurokawa et al. (1986) administered 0, 250 or 500 ppm sodium chlorite (0, 36 or 71 mg ClO_2-/kg-day) in drinking water to B6C3F1 mice of both sexes for 85 weeks, increased liver tumors (hyperplastic nodules or hepatocellular carcinomas) and lung adenomas in males were found relative to concurrent controls, but not historical controls. Similarly, Yokose et al. (1987) did not show clear evidence of carcinogenicity in B6C3F1 mice administered 0, 250 or 500 ppm of chlorite for 80 weeks. Application of 100 mg ClO_2-/kg twice weekly for 51 weeks to the skin of female SENCAR mice did not induce skin tumors. However, application of chlorite to skin following a single dermal application of DMBA induced skin tumors, primarily squamous cell carcinomas, in 6/20 treated vs. 0/20 controls (Kurokawa et al., 1984).

As reviewed in U.S. EPA (1994) concentrates of chlorine dioxide-treated drinking water generally did not induce a positive mutagenic responses in bacterial systems nor did chlorine dioxide or chlorite induce clastogenic activity in mammalian assays (micronucleus test, sperm head morphology or bone marrow chromosomal aberrations).

In accordance with the 1986 cancer guidelines, chlorine dioxide and chlorite were categorized in Group D, *Not classifiable as to human carcinogenicity* (U.S. EPA, 1986). In accordance with the 1996 proposed cancer guidelines, the carcinogenicity of these chemicals are considered *Cannot be determined* (U.S. EPA, 1996b).

2.3 Other Studies

The criteria document presented numerous studies examining the role of chlorite in producing oxidative damage to the erythrocyte and methemoglobin formation. The study by French et al. (1995) is a comparative study among several methemoglobin-forming compounds, but does not shed any new light on mechanistic information.

French et al. (1995) examined the *in vitro* methemoglobin-forming potentials of chlorite in Dorset sheep erythrocytes, and compared it with four other direct-acting methemoglobin-forming agents in the same system. Samples were incubated for 1 hour with doses of 0.0005, 0.005, 0.01 or 0.02 mM chlorite. Chlorite was ranked as among the least potent agents compared to *p*-dinitrobenzene, *o*-dinitrobenzene, copper, and nitrate.

2.4 Synthesis and Evaluation of Major Noncancer Effects and Mode of Action

No subchronic or chronic studies adequately assess the systemic toxicity of chlorine dioxide. The study by Haag (1949) identifies a NOAEL of 1.3 and a LOAEL of 13 mg/kg-day for chlorine dioxide, but is not considered of adequate design. For chlorite, one adequate subchronic study (Harrington et al., 1995a) identified a NOAEL of 7.4 mg/kg-day and a LOAEL of 18.6 mg/kg-day based on effects on spleen and adrenal weights and histopathologic changes in the gastric mucosa. However, the NOAEL identified by Harrington et al. (1995a) is higher than the LOAEL for developmental/neurodevelopmental effects (6 mg ClO_2^-/kg-day) identified by CMA (1996). Mechanisms or mode(s) of action by which chlorine dioxide and chlorite exert toxic effects are largely not understood.

2.4.1 Major Noncancer Effects

Developmental and neurodevelopmental effects are the critical effect(s) for both chlorine dioxide and chlorite. A consistent finding is alteration in pup brain weight and neurobehavioral endpoints. These effects tend to occur at dosages greater than 10 mg/kg-day for chlorine dioxide and 6 mg/kg-day for chlorite (See Figures 1 and 2).

NOAEL of 3 mg/kg-day (20 ppm in drinking water) and a LOAEL of 14 mg/kg-day (100 ppm) for chlorine dioxide were identified in rats based on decreased thyroid hormone levels, and decreased exploratory, locomotor and running wheel behavior in pups after treatment of dams from 2 weeks prior to breeding, through gestation and lactation (Orme et al., 1985). A LOAEL of 14 mg/kg-day (100 ppm in drinking water), based on decreased brain weight, brain cell number and protein content, and decreased exploratory behavior in pups, was identified in rats in several studies of chlorine dioxide

Figure 1. Chlorine Dioxide: Developmental Studies

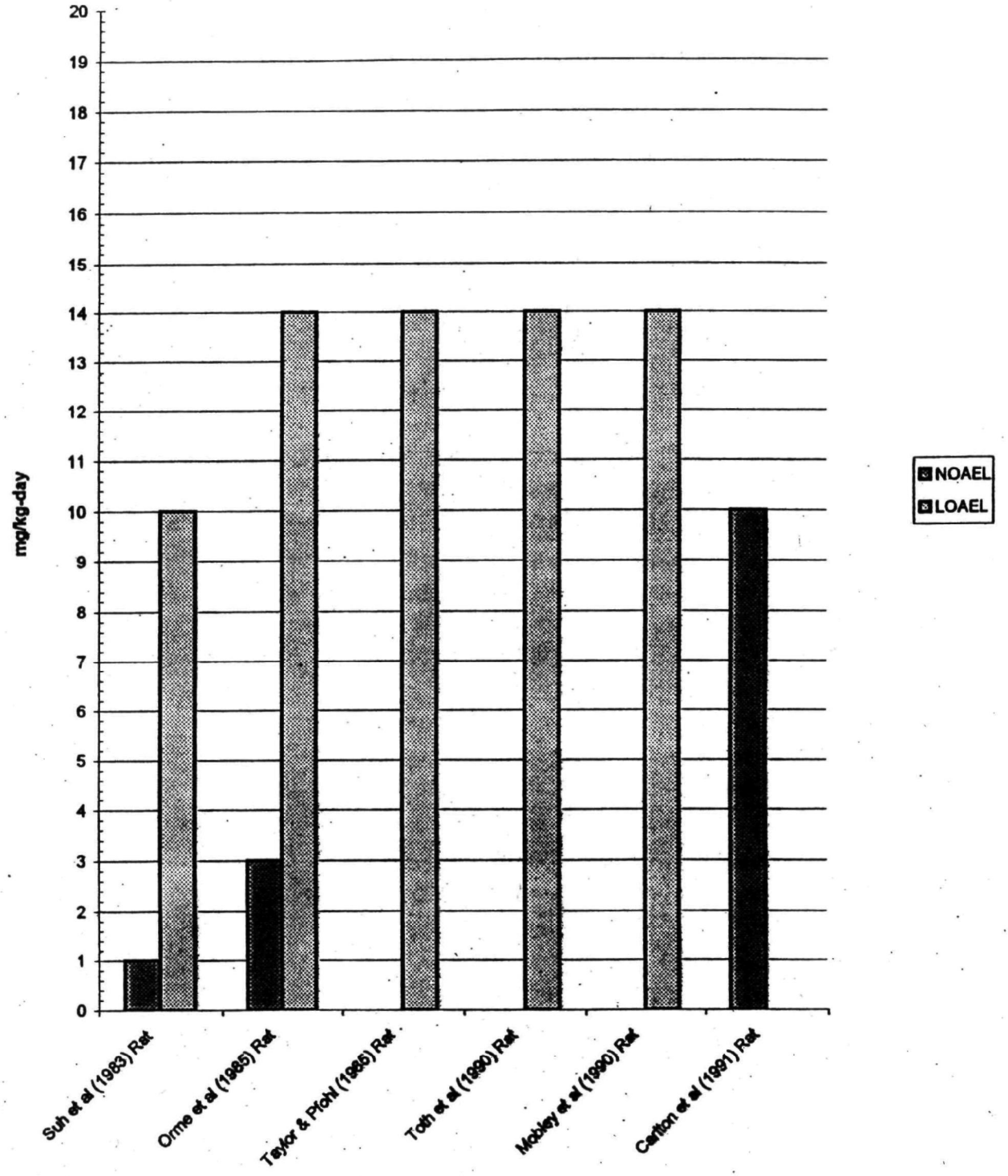
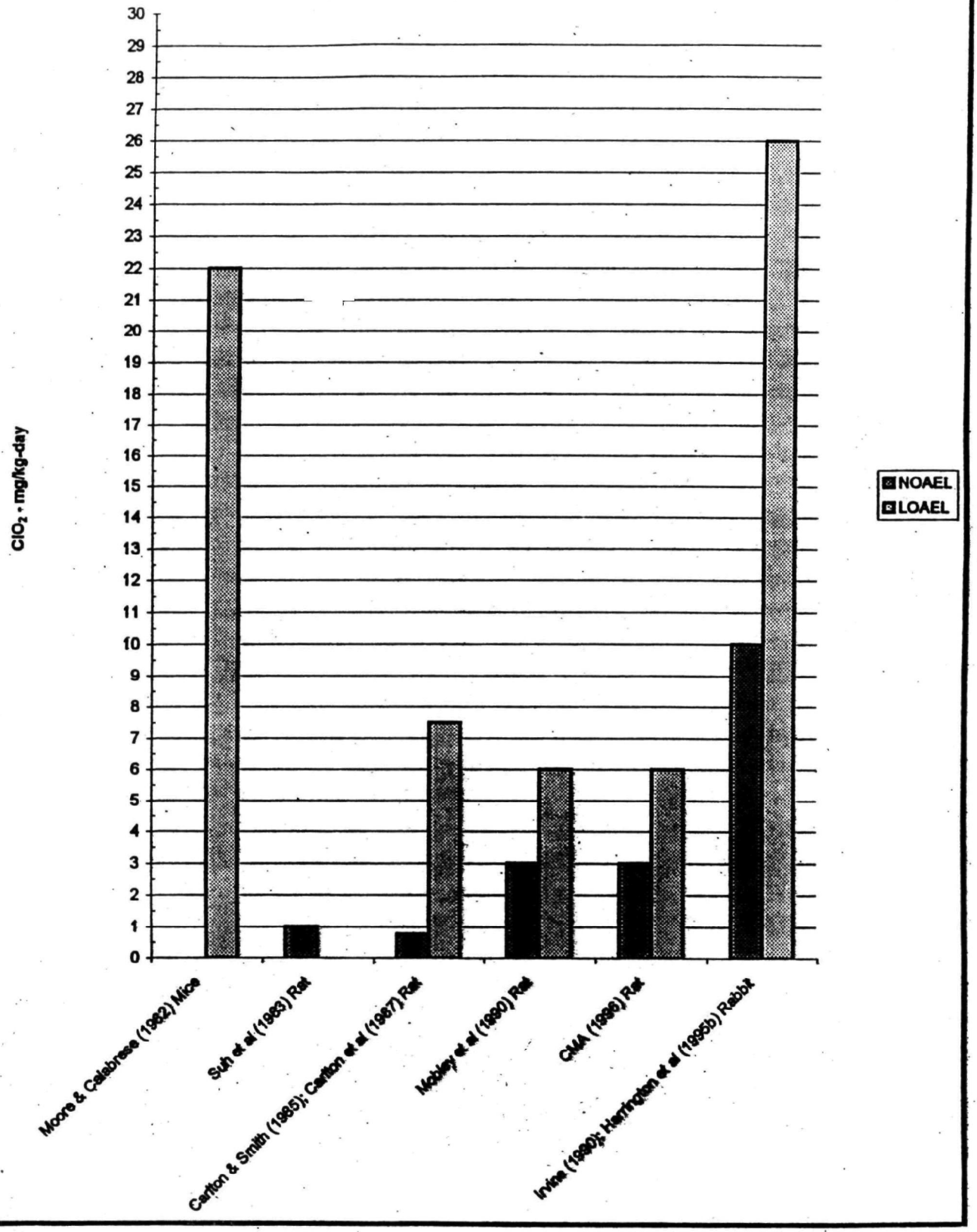


Figure 2. Chlorite: Developmental Studies



administered on similar breeding. gestation and/or lactational regimens (Taylor and Pfohl, 1985: Toth et al., 1990. Mobley et al., 1990). Collectively these studies formed a weight of evidence basis for the previous derivation of the RfD (U.S. EPA, 1994). Carlton et al. (1991) reported 10 mg/kg-day as a NOAEL for reproductive parameters and thyroid hormone levels in pups in a study employing gavage treatment of rats of both sexes prior to mating, and females throughout gestation and lactation. Suh et al. (1983), however, reported a LOAEL at 10 mg/kg-day for decreases in live births and number of implants in rats drinking water for approximately 75 days and then during gestation.

For chlorite, a LOAEL of 3 mg/kg-day for decreased exploratory activity from Mobley et al. (1990), supported by similar behavioral and neurohistopathology changes from the chlorine dioxide studies (Orme et al., 1985; Taylor and Pfohl, 1985; Toth et al., 1990), was used as the basis of the previously-derived RfD (U.S. EPA, 1994). The 3 mg/kg-day dose in Mobley et al. (1990) showed small changes, whereas changes observed at 6 mg/kg-day were more consistent with findings from several other studies. Similarly, lowered auditory startle response, reduced absolute brain weight, and reduced liver weight were observed at 5.9 mg ClO_2^-/kg-day (LOAEL), but not at 2.9 mg ClO_2^-/kg-day (NOAEL), in rats in a 2-generation study (CMA, 1996). Adverse effects in developmental studies in other species occur at higher doses. A LOAEL of 22 mg/kg-day (100 ppm chlorite in drinking water) was identified in mice based on a 14% decrease in pup weight after treatment of dams through gestation and lactation; a NOAEL was not identified (Moore and Calabrese, 1982). Irvine (1990); Harrington et al., (1995b) showed decreased fetal weight and delayed ossification in rabbit pups exposed to 26 mg/kg-day (LOAEL) but not to 10 mg/kg-day (NOAEL).

Data for reproductive endpoints for both chemicals are not as consistent, but suggest there may be alterations in male reproductive toxicity. These data, however, do not clearly define a threshold or NOAEL/LOAEL boundary. At 1.0 mg/kg-day (10 ppm in drinking water), for both chlorine dioxide and chlorite, reduced incorporation of radiolabeled thymidine into testicular DNA after a 3- month treatment was identified in male rats (Abdel-Rahman et al., 1984b). Suh et al. (1984) showed decreased testicular DNA synthesis in rats administered 1 mg/kg-day chlorine dioxide. These studies are not particularly useful for risk assessment without concomitant functional endpoints of male reproductive toxicity. While Carlton and Smith (1985) and Carlton et al. (1987) identified decreased sperm motility and increased abnormal sperm morphology in male rats exposed for 72-76 days to 7.5 mg ClO_2^-/kg-day (LOAEL), but not 0.75 mg/kg-day (NOAEL), CMA (1996) showed no alterations in sperm indices (motility, concentration, morphology) up to 22 mg/kg-day. Meier et al. (1985) showed no alterations in sperm morphology in mice for either chemical up to 16 mg/kg-day by gavage for 5 days.

2.4.2. Mode of Action

The drinking water criteria document (U.S. EPA, 1994) discussed possible modes of action whereby ClO_2 and ClO_2- produce hematological and systemic effects. The mechanism(s) are still incompletely understood.

Oxidative damage to the erythrocyte and production of methemoglobin are most likely related to their properties as oxidants (U.S. EPA, 1994). Chlorite is thought to be the intermediate species responsible in many of the hematological effects of chlorine dioxide because of its more efficient production of methemoglobin, depletion of RBC glutathione and alteration of erythrocyte fragility.

In a series of experiments, Bercz and coworkers (Bercz et al., 1982, 1986; Harrington et al., 1985) have suggested that chlorine dioxide increases binding of dietary iodide to gastrointestinal tissue and contents, producing a functional iodide deficiency. Bercz et al. (1982) found decreased levels of circulating thyroxine in monkeys drinking water containing > 9.5 ClO_2 mg/kg-day, but not 44 mg ClO_2-/kg-day, for 4-6 weeks. In a follow-up study, Harrington et al. (1986) demonstrated increases in thyroid iodide uptake and a rebound in thyroxine levels in monkeys 1 year after an 8 weeks exposure to approximately 5 mg ClO_2/kg-day in drinking water. Unlike monkeys, rats showed dose-related declines in thyroxine levels and no alteration in thyroid iodide uptake following an 8-week exposure to 10 mg ClO_2/kg-day in drinking water.

Whether either, or both, of these mechanisms are operable in inducing reproductive, developmental and neurodevelopmental effects is not known. One could also speculate that hypothyroidism, induced by chlorine dioxide-alteration of iodide uptake in the gastrointestinal tract, might contribute to alterations in maternal or neonatal behavior. Alternate, as yet unknown, mechanisms are also plausible as little definitive mechanistic data are available. Additional research is needed to understand how chlorine dioxide and chlorite induce alterations in fetal/neonatal neurodevelopment and behavior.

2.5 Hazard Assessment Issues

2.5.1 Strengths and Weaknesses of Evidence

Epidemiologic information comes from community ecological studies and has been fraught with methodological problems, such as the lack of characterization of exposure to other agents in the drinking water and controlling for confounding factors. These studies do little to confirm a possible association between exposure to chlorine dioxide and chlorite and adverse reproductive or developmental outcome in humans.

The animal toxicity data base for chlorine dioxide and chlorite are fairly comprehensive, comprised of subchronic and chronic studies, reproductive and developmental studies, and toxicokinetic and mechanistic information.

Multiple animal studies have shown similar alterations in neurodevelopmental endpoints, such as brain weight and behavioral measures. The majority of these studies have used sufficient numbers of animals and employed routes of exposure (gavage and drinking water) relevant to human exposure. The majority of the developmental studies have utilized rats and have shown a fairly consistent definition of the NOAEL/LOAEL. For chlorine dioxide, all the developmental studies were conducted in rats. For chlorite, there is one developmental study in mice (Moore and Calabrese, 1982), one developmental study in rabbits (Irvine, 1990; Harrington et al., 1995b), and numerous developmental studies in rats.

Reproductive studies in male animals are not consistent in demonstrating alterations in spermatogenic indices, i.e., abnormal morphology or motility; however, reported effects seem to appear at doses higher than the adverse developmental effects. Similarly, clinically or toxicologically significant alterations in hematological parameters occur at higher doses.

The mode of action for induction of adverse neurodevelopmental effects is not known. It is also not known whether the rat is an adequate model for toxicity of chlorine dioxide and chlorite in humans. However, this species is widely used to characterize reproductive and developmental effects in humans.

2.5.2 Susceptible Populations

No subpopulation is recognized as uniquely susceptible to chlorine dioxide and chlorite exposure. Developmental effects were noted in animal studies following both *in utero* and postnatal exposure, suggesting infants and children may be more likely than adults to experience adverse effects following exposure to chlorine dioxide and chlorite, although the reasons for this increased sensitivity are not fully understood. It is well-recognized that neurological development continues after birth and that gastrointestinal uptake of many nutrients and chemicals is greater in the neonate than the adult.

3.0 Dose-Response Assessment (RfD Derivation)/Characterization

3.1. Choice of Principal Study and Critical Effect

The CMA (1996) two-generation reproduction study was selected as the critical study for the development of an RfD for both chlorine dioxide and chlorite. Since chlorine dioxide readily degrades to chlorite, the critical effect(s) and dose at which those effects are seen are similar. Therefore, the toxicity information for chlorite is relevant to deriving the RfD for chlorine dioxide. The CMA (1996) rat study was designed to evaluate the effects of chlorite (sodium salt) on reproduction and pre- and post-natal development when administered orally via drinking water for two successive generations. Developmental neurotoxicity and hematological toxicity were also evaluated in this study.

Sodium chlorite was administered at 0, 35, 70, and 300 ppm via drinking water to male and female Sprague Dawley rats (F0 generation) for ten weeks prior to mating. Dosing continued during the mating period, pregnancy and lactation. Reproduction, fertility, clinical signs, and histopathology was conducted on F0 and F1 males and females. F1 and F2 pups were evaluated for growth and development, clinical signs, and histopathology. In addition, animals from each dose group in the F1 were allocated for neurotoxicity assessment (e.g., neurohistopathology, motor activity, learning ability and memory retention, functional observations, auditory startle response). Limited neurotoxicological evaluations were conducted on F2a and F2b pups.

Alterations in multiple endpoints define the LOAEL-NOAEL boundary in the CMA (1996) study. The effects observed included statistically significant decreases in pup body weight, absolute brain weight, pup survival, liver weight, and lowered startle amplitude at the 300 ppm dose. Statistically significant decreases in auditory startle amplitude, liver weight and absolute brain weight occurred at 70 ppm. Although different responses were found for auditory startle (as indicated by measures of amplitude, latency and habituation), this is not unexpected given that these measures examine difference aspects of nervous system function, and thus can be differently affected. Transient alterations in neurofunctional (or neurochemical) measures, such as in the auditory startle response, can occur without neuropathological changes, and are considered of neurotoxic concern (U.S. EPA, 1997a). Some of effects observed at 70 and 300 ppm occurred in both sexes and in more than one generation, and are considered toxicologically significant, which is consistent with U.S. EPA guidelines for reproductive, developmental, and neurotoxicity risk assessment (U.S. EPA, 1991, 1996a, 1997a). The NOAEL for this study is 35 ppm (2.9 mg ClO_2^-/kg-day) and the LOAEL is 70 ppm (5.9

mg ClO_2-/kg-day) based on lowered auditory startle amplitude, decreased absolute brain weight and decreased liver weight.

While the CMA (1996) study is adequate and conducted with sufficient numbers of animals of both sexes at multiple dose levels showing a range of effects, and examined numerous endpoints, there are several limitations. Lack of pair-watered and pair-fed control animals confounds the results and precludes making definitive conclusions as to whether the alterations in food and water consumption and body weight are related to water palatability or a direct toxic effect of agent. Discontinuation of exposure for the animals undergoing neurotoxicity testing limits the likelihood of finding a positive effect, precludes comparison of the data with that of other rats with continued exposure, and does not reflect the expected lifetime exposure by humans to these chemicals in drinking water. In addition, a lack of detailed description of experimental methods and positive control data (including estimates of variability), and in some cases, inappropriate statistical analysis, limit interpretation of the neurobehavioral tests.

The principal study is supported by the developmental studies by Orme et al. (1985), Taylor and Pfohl (1985), Toth et al. (1990) and Mobley et al. (1990) wherein rats were administered chlorite or chlorine dioxide at similar dosages in drinking water also showed alterations in exploratory and locomotor behavior and reduced brain weights (NOAELs of 3 mg/kg-day; LOAELs of 6-14 mg/kg-day).

The RfD is derived for chlorine dioxide and chlorite from the NOAEL from the CMA study (1996) of 2.9 mg ClO_2-/kg-day. This dose was determined from the nominal water concentration based on measured water consumption and adjusted for the molecular weight of the salt, so that doses are expressed as the chlorite ion. (For example, males administered 35 ppm, had intakes of sodium chlorite equivalent to 3.9 mg/kg-day. Adjusting for the molecular weight of sodium chlorite [MW= 90.5] relative to the chlorite ion [MW= 67.5], gives the NOAEL dose of 2.9 mg ClO_2- /kg-day).

3.2 Oral Reference Dose Derivation

An uncertainty factor of 100 is appropriate for developing the RfD for chlorite and chlorine dioxide. This composite factor includes a factor of 10 to account for uncertainties associated with inter-species (extrapolating from experimental animals to humans) and a factor of 10 for differences in response to toxicity within the human population (to protect sensitive subpopulations). Because the critical effect is a developmental effect in a database that includes chronic studies, it is not necessary to use an uncertainty factor to account for use of a less-than-lifetime study. A default modifying factor of 1 is applied.

In derivation of the RfD in U.S. EPA (1994), an uncertainty factor of 3, in addition to the composite 100 for inter- and intra-species variability, was applied for chlorine dioxide to account for a database deficiency due to the lack of a multigenerational reproductive study. For the derivation of the RfD for chlorite an uncertainty factor of 10, to account both for the same data base gap (lack of a multigeneration reproductive study) and extrapolation from a LOAEL to a NOAEL, was applied in addition to the composite 100 for inter- and intra-species variability. These uncertainty factors are no longer needed since the CMA study (1996) fills the database gap for a multigenerational study and the chlorite RfD is now based on the NOAEL from CMA (1996).

The RfD is obtained by rounding the NOAEL of 2.9 mg/kg-day to 3 mg/kg-day, then dividing by the product of the uncertainty factors and the modifying factor (100). The resulting RfD is 0.03 mg/kg-day for chlorine dioxide as well as for chlorite.

The RfD of 0.03 mg/kg-day is considered to be protective of susceptible groups, including children, given that the RfD is based on a NOAEL from a two-generation reproductive rat study. A two-generation reproductive study evaluates the effects of chemicals on the entire developmental and reproductive life of the organism. Additionally, current methods for developing RfDs are designed to be protective for sensitive populations. In the case of chlorine dioxide and chlorite, an additional factor of 10 was used to account for variability between the average human response and the response of more sensitive individuals.

4.0 Risk Characterization Summary

Confidence in the critical study, CMA (1996) is medium. Although the study design and analytical approaches are consistent with U.S. EPA testing guidelines, some limitations in the design and conduct of the study exist. Confidence in the database is high as there are studies in multiple species, chronic duration studies in males and females, reproductive/developmental toxicity studies and a multigenerational study. The threshold for adverse effects is consistently defined amongst the animal studies. Resulting confidence in the RfD is medium-to-high.

Remaining areas of scientific uncertainty include the mode of action of chlorine dioxide and chlorite in producing adverse effects on multiple organ systems, including reproductive, developmental, and hematological effects. Inherent in the uncertainty with the mode of action is identification of the susceptible populations or subgroups, since additional research in this area would aid in better quantifying the additional risk to these groups. Well-designed and conducted epidemiological studies in communities with drinking water disinfected with these chemicals would decrease uncertainty in the utilization of animal models for determination of human health effects.

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APPENDIX: SUMMARY OF REVIEWERS' COMMENTS

Statement of Work

TITLE: Peer review of Health Risk Characterization Report on Chlorite

BACKGROUND:

The mission of the United States Environmental Protection Agency's (EPA) Office of Water (OW) is to protect public health and the environment from adverse effects of contaminants in media such as ambient water, drinking water, waste water, sewage sludge and sediments. This procurement relates to the peer review of a health risk assessment on the disinfection by product, chlorite. This risk assessment will be used in support of EPA's stage 1 disinfection by product rule which is scheduled to be final in November 1998. The Safe Drinking Water Act Amendments of 1996 emphasize that "the best peer review science" be used in carrying out SDWA regulations.

PURPOSE:

A cancer risk assessment/characterization document has been recently prepared that cites and updates EPA's 1994 assessment on chlorite. This 1998 document considers a new study by the Chemical Manufacturers Association on the reproductive and neurodevelopmental effects of sodium chlorite.

TASK DESCRIPTION:

This purchase will procure a peer review on the 1998 EPA chlorite risk assessment report. EPA has attached the 1998 chlorite risk assessment document, consisting of approximately 20-25 pages, to be reviewed (Attachment 1), as well as supporting materials; EPA's 1994 Criteria Document on chlorite (Attachment 2), and a summary of the CMA study with the accompanying peer review comments (Attachment 3). The peer reviewer shall submit written comments that are clear/transparent, and constructive. They shall comment on whether the document clearly and adequately discusses:

- the weight of the evidence
- the critical effect
- the key lines of evidence
- uncertainties in the risk assessment
- scientific basis for the derivation of the reference dose

The peer reviewers shall indicate where they are in agreement with the report and where they disagree. If they disagree with any part of the report or find a weakness in the report, they shall recommend explicit guidance on revising the report. They shall provide comments that include an overall general summary on the acceptability and adequacy of the risk assessment, and specific comments as needed (comment one on page X, paragraph X, line X).

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Summary of External Reviewer Comments
February 1998 Draft Chlorine Dioxide/Chlorite Risk Characterization

September 1998
Patricia McGinnis

Reviewers: 1= Foster
2= Gandy
3= Tyl

Comments below are paraphrased from the reviewers' comments.

Editorial revisions were made in response to comments by the reviewers.

Introduction

1. Comment (1): A reference is needed for the statement that chlorine dioxide dissociates under aqueous conditions and in the GI tract of animals; this is a key assumption.

Response: Reference (DWCD, 1994) added.

Hazard Assessment

2. Comment (1): It is well understood that these agents can cause methemoglobin, etc. (hematological effects). The available human data on hematological parameters should be included.

Response: Section 2.0 revised to clarify hematological effects data for short-term exposure. Reference to the DWCD (U.S. EPA, 1994) was added.

3. Comment (3): The status of the referent "neighboring" community as not exposed to chlorite, or any drinking water disinfectant, should be made explicit.

Response: comment refers to Kanitz et al. (1996) --In section 2.1, clarified that the community used water pumped from wells without any water disinfection treatment.

4. Comment (1, 3): The section (Section 2.2.2.1) describing the nasal lesions and potential confounding with direct exposure to drinking water could be expanded to explain why this potential artifact of treatment was noted. A fuller description of the nasal lesion could convince the reader whether inadvertent treatment to the nasal passages was likely to happen; this is an important point to place these lesions in perspective for risk assessment.
Response: Comment refers to Daniel et al. (1990). Authors do not provide further detail of the nasal lesions. The sentence was clarified to the extent possible based on the authors' discussion.

5 Comment (1) In Table 1, additional comments should be made to the endpoints column to clarify why the observations are considered adverse

Response: Table 1 summarizes systemic toxicity data and lists the NOAEL and LOAEL. Endpoints listed are for the LOAEL (therefore, considered adverse), unless no LOAEL is stated. In the case where there is no LOAEL, the endpoint examined is the NOAEL and the column states "not dose-dependent" or "no effects on ..." to clarify the non-adversity of the endpoint.

6. Comment (1): Was water consumption presented in the Harrington study? A presentation of water consumption data or a summary statement is important because there is a tremendous physiological demand for fluids during pregnancy.

Response: This comment refers to the Harrington et al. (1995b) rabbit teratology study. The study description includes a summary statement about alterations in water consumption; data were not shown by the authors and additional details cannot be provided.

7. Comment (1): Why is significant growth retardation in the 26 and 40 mg/kg/d groups (in Harrington et al., 1995b) not considered adverse? An alteration of growth, even in the presence of maternal toxicity, should be considered adverse according to the guidelines.

Response: Results are considered adverse. Study text was revised to better explain the results for the fetuses and to show the LOAEL at 26 mg/kg/d for maternal and fetal endpoints.

8. Comment (3): It should be clarified as to which sexes and doses as well as when reductions in water consumption, food consumption and body weight gain were observed (in the CMA, 1997, study) so that palatability and toxicity may be discerned.

Response: Clarification added in second CMA paragraph, section 2.2.2.2- various times in both sexes in all generations. Too cumbersome to add all that data and its not the point of risk characterization document, particularly when these endpoints are not the critical effect.

9. Comment (3): It should be confirmed that the NOAEL is 35 ppm for sodium chlorite, since the purity of the test material was presented as 81.4%. Did the study authors make an adjustment to the concentration of the test material in the drinking water to compensate for the purity or was the calculated intake (in mg/kg/day) corrected for purity? The former may have contributed to the observed "palatability problems" (especially the sodium component).

Response: Apparently the study authors adjusted the concentration of sodium chlorite to compensate for the purity of the test material- clarification added to first CMA paragraph, section 2.2.2.2.

10. Comment (1): The statement regarding the CMA (1997) study that discontinuation of exposure for animals undergoing neurotoxicity testing minimizes the likelihood of finding a positive effect and precludes comparison is perplexing. The guidelines indicate that exposure should be from gestation day 6 until postnatal day (PND) 10 with examination postexposure to PND 60, and that offspring from a multigeneration study is acceptable. The study meets the

requirements and can be appropriately compared with others; positive control data were provided

Response. Additional text added to discussion of limitations of the CMA study to clarify this statement (third paragraph on CMA study, section 2.2.2.2). While positive control data provided, still some limitations (see next paragraph).

11. Comment (1): The statement (Section 2.2.3) that carcinogenicity data from animal studies are largely inadequate should be qualified, as the mouse study in the second paragraph does not seem to have been taken into consideration.

Response: The statement in the first paragraph referred to chlorine dioxide; there are no long term bioassays for chlorine dioxide. The mice in the 85-week study, described in the second paragraph, were administered sodium chlorite. Introductory sentence added at beginning of second paragraph referring to chlorite to clarify for reader.

12. Comment (3): The difference in sperm morphology outcomes between the two studies may be due to differences in study duration as well as species.

Response: refers to last paragraph, section 2.4.1, Carlton and Smith (1985), Carlton et al. (1987) and Meier et al. (1985). Sentences reworked- CMA male rat results added and compared with Carlton studies. Meier mouse study sentence revised.

13. Comment (1): The statement about the same route and dosage for the rat and rabbit studies during the same period of organogenesis is important since often the exposure period is of equal importance to dose level in developmental studies.

Response: refers to section 2.5.1. Section revised because Irvine (1990) and Harrington et al. (1995b) found to be same study and NOAEL/LOAEL for Harrington 1995b clarified (see comment re: Harrington study above).

14. Comment (2): The identification of the last two potentially susceptible populations seems speculative because it is based on hypothetical mechanisms. The level of confidence for identifying these subpopulations as uniquely susceptible is very low.

Response: Refers to Section 2.5.2. The point is well-taken. Section was revised.

18 Comment (1). Was the whole reproductive and developmental data considered adequate for risk assessment? Should additional (uncertainty) factors (UF) be invoked for "child health issues"? This reviewer believes these are adequate and th·· a further UF is not required.

Response: Yes- whole database considered and with new 2-generation study (CMA, 1997), there are no database gaps. No further UF needed. Additional explanation was added 4th paragraph, section 3.2.

19. Comment (2, 3): The approach for RfD calculation is valid and appropriate. The UF of 100 is appropriate (the UF of 3 is no longer needed). (However, one of the reviewers disagrees with NOAEL -see comment above).

Response: agree

Risk Characterization Summary

20. Comment (1): Can confidence in the CMA study be increased upwards from medium (see comment above on neurotoxicity)?

Response: No, there were limitations in study design and conduct that preclude upgrading confidence.

21. Comment (3): Reviewer concurs with "medium" confidence in the critical study (CMA, 1997) in recognition of the limitations to study design, performance and final report presentation. Reviewer agrees with "medium- to-high" confidence in the RfD based on the database.

Response: agree

Dose-Response (RfD Derivation)/Characterization

15 Comment (1, 3): Report clearly discusses the strengths and weaknesses of the weight of evidence; reviewer concurs with critical study and the NOAEL of 35 ppm.

Response: agree

16. Comment (3): The critical study (CMA, 1997) should be considered only "adequate" as there were problems with the study design and conduct. The reviewer considers the following as some of the problems: purity (low) of the test material; (presumed) method of correcting for test material purity; discontinuation of exposure after weaning and testing of pups on PND 60; lack of evaluations of F2a males and females for preputial separation; lack of collection of coagulating gland weight; and in assignment of 300 ppm as reproductive and developmental NOEL and 70 ppm as hematological NOAEL, the laboratory has discounted their data without explanation.

Response: section 3.1 , 4th paragraph. Revised to state study adequate; agree. NOAEL and agree with reviewer that there are limitations with the study (some so noted above by reviewer and others also noted in the text of section 3.1).

17. Comment (2): Choice of the reproductive/developmental endpoint and critical study is appropriate but NOAEL and LOAEL should be 70 and 300 ppm, respectively, not 35 ppm and 70 ppm. A treatment-related decrease in offspring viability is not supported; variations were small. The NOAEL should be 300 (or possibly 70 ppm), not 35 ppm, for offspring viability. Significant differences in offspring body weight were detected only in 300 ppm of either F1 or F2 generations; LOAEL should be 300 ppm and NOAEL 70 ppm for offspring body weight. There is no convincing evidence that a treatment-related, biologically relevant, adverse effect was measured in auditory startle amplitude. (The reviewer constructed tables for males and females for PND 24). The results for maximum amplitude of response are inconsistent, the effect of maximum startle amplitude was transitory (there was no effect on PND 60), and there was no supporting histopathological evidence. The interpretation of the auditory startle-habituation test results seems equivocal, at best. There is no evidence for a NOAEL of 35 ppm based on "possible" alteration in learning behavior. Likewise, any small decrease in absolute brain wet weights are more likely a function of smaller body weight rather than a treatment-related effect to the CNS. An argument can be made that the effects in treated rats are most likely due to decreased food and water intake due to altered palatability. A NOAEL of 70 ppm is prudent and protective, since the true value is likely higher. More description and justification is needed for the selection of the NOAEL and LOAEL.

Response: Agree with lack of treatment-related effect in offspring viability and body weight-sections 2.2.2.2 CMA study and 3.1 revised. Appreciate reviewer's analyses of data but disagree with reviewer- there is an adverse effect on auditory startle, possibly learning and absolute brain weight. Section 3.1 expanded to explain transient alterations and different responses for auditory startle are still considered indicative of toxicity and adverse effects.

Peer Review of the Draft Document Entitled
"Health Risk Assessment/Characterization of the
Drinking Water Disinfectant Chlorine
Dioxide and the Degradation By-product Chlorite"
Dated February 6, 1998

Prepared for
Health and Ecological Criteria Division
Office of Science and Technology
Office of Water
U. S. Environmental Protection Agency
Washington, DC 20460
By Toxicology Excellence for Risk Assessment (TERA)
Cincinnati, Ohio 45223

I have received and examined the following documents:

1. The draft document specified above under review
2. External peer review of CMA Study 2-Generation (October 9, 1997)
3. Response to review comments by CMA Chlorine Dioxide Panel (January 26, 1998)
4. Volume 1 (of 6) of the study entitled "Sodium Chlorite: Drinking Water Rat Two-Generation Reproductive Toxicity Study" (March, 1996) performed by Quintiles England, Ltd., for the Chloride Dioxide Panel of CMA, Quintiles Report Reference No. "CMA/17/96" (and a separate copy of title page [p1] and summary pp. 25-27])
5. The charge to the Peer Review Panel by Dr. Vicki Dellarco, U.S. EPA (dated February 4, 1998)
6. Final Draft for the Drinking Water Criteria Document on Chlorine Dioxide, Chlorite and Chlorate (dated March 31, 1994), prepared for Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, U.S. EPA, Washington, DC, by Clement International Corporation.

The peer reviewers have been asked to critique the draft risk assessment document in terms of (1) the weight of the evidence, (2) the critical effect, (3) the key lines of evidence, (4) uncertainties in the risk assessment, (5) the scientific basis for the derivation of the reference dose, as presented in the document, and (6) the quality of the risk assessment document, and (7) the quality of the critical study (CMA two-generation).

The comments of this reviewer, on whether the document clearly and adequately discusses the seven aspects specified above, and whether she agrees or disagrees with the report, are as follows.

1. <u>The Weight of the Evidence</u>

   The report clearly discusses the strengths and weaknesses of the weight of the evidence. I concur with the assessment as presented throughout the report, and especially on p. 24 and following.

2. The Critical Effect

I agree with the report (Section 3.0, pages 25 and following) that the critical study is the CMA (1996) two-generation study of sodium chlorite in the drinking water in rats. I concur with the discussion in the report on its strengths and weaknesses (report p. 26, last paragraph). I would request confirmation from the authors of the study that the NOAEL of 35 ppm is in fact sodium chlorite, since the purity of the test material was presented as 81.4% (Study Report Volume 1, p. 29). The impurities are presented in Volume 1 of the Study Report, on page 118, and the text of the study report (p. 31) indicates "An adjustment for purity was made using a conversion factor of 1.23." I interpret this to mean the concentration of the test material in the drinking was increased to compensate for the purity, rather than that the calculated intake in mg/kg/day was corrected for purity. The increased amount of test material (especially the sodium component) may have contributed to the observed "palatability problems." The report assesses the critical study as "well-designed... and acceptable" (p. 17). This reviewer would assess the study only as "adequate."

I concur with the report that the NOAEL for developmental effects is 35 ppm, equivalent to 2.9 mg ClO_2^-/kg body weight/day.

3. The Key Lines of Evidence

I concur with the report discussion on the key lines of evidence, building on the Final 1994 Draft of the Drinking Water Criteria Document on Chlorine Dioxide, Chlorite and Chlorate, and using the CMA study as the critical study.

4. Uncertainties in the Risk Assessment

The report indicates that the confidence in the critical study is "medium" (report, p. 78), and indicates limitations in the design and conduct of the CMA two-generation study. I concur with the confidence level and with the recognition of limitations to study design, performance and (in my opinion) final report presentation (see this reviewer's item no. 7).

The report also indicates that the confidence in the RfD is "medium-to-high." Based on the database, I concur.

5. Scientific Basis for the Derivation of the Reference Dose

The report uses the 2.9 mg/kg/day chlorite, the NOAEL from the two-generation study, and applies the standard default uncertainty factors of 10 (interspecies extrapolation from rats to humans) x 10 (intraspecies extrapolation to protect sensitive subpopulations) x 1 (modifying factor, as it was deemed unnecessary to account for use of a less-than-lifetime exposure since the critical effect was a developmental effort). Therefore, the report rounds the 2.9 mg/kg/day to 3 mg/kg/day, divides by 100 (10 x 10 x 1) to obtain an RfD (reference dose) of 0.03 mg/kg/day "for chlorine dioxide as well as for chlorite." (p. 27).

This derivation is standard, relatively conservative and uses accepted default values for uncertainty factors. I concur with the basis, approach and conclusion.

6. Quality of the Risk Assessment Document

The document is adequate. There are many typographical errors, some trivial, some substantive, and some areas when the logic trail/discussion should be explicit, not implicit. Examples follow:

A. Typographical errors

i. Table 1, page 7, Revis *et al.* (1986) doses were 0, 0.07 and 0.5 (not 0, 0.7 and 0.5) Fix endpoints to line up with sexes in Daniel *et al.* (1990) reference.

ii. Table 2, page 8, Revis *et al.* (1986) doses were 0, 0.07 and 0.5 (not 0, 0.7 and 0.5)

iii. Page 5, first full paragraph, sex ratios are usually expressed as > or < 1.0, *i.e.*, 0.86 versus 1.12 (not 86 versus 112)

iv. Page 5, line 11, "...contained any chemical..." (not "contained and chemical...")

B. Logic Trail/Discussion not explicit

i. Page 4, Section 2.1, line 13. The status of the referent "neighboring community" as not exposed to chlorite should be made explicit. Were they exposed to any drinking water disinfectant?

ii. Page 4, Section 2.2.2.1, lines 10-14. Statement that the nasal lesions were "most likely the result of direct contact with the chlorine dioxide solution rather than ingestion of the drinking water" and may be an "artifact of treatment" needs some additional explanatory text.

iii. Section 2.2.2.2, page 16, last paragraph, lines 1-3. The "reductions in water consumption, food consumption and body weight gain in all generations" should be identified as to which sexes, which doses, and when the effects were observed. This reviewer's concern is that the typical pattern of palatability problems with rats (from dosed feed or dosed water) is usually initially low intake values which rise over time as the animals adjust (rats are awesomely practical animals), versus the pattern of toxicity which is usually initially normal intake values which drop over time as, *e.g.*, body weights/weight gains drop, and/or other symptomology appears which is characteristic of the toxicity. (Volume 1 of the study report does not include this information.)

iv. Figure 2. Suggest cluster the bar graphs by species, *i.e.*, rat, mouse, rabbit, and in accompanying text on p. 22 also suggest group by species.

v. Section 2.4.1, top page 23. The difference in sperm morphology outcomes between the two studies may be due to differences in study duration (72-76 days with effects versus 5 days with no effects) as well as to differences in species (rat versus mouse).

vi. The CMA study is routinely indicated as "the CMA (1996)" (*e.g.*, p. 26 and 27); it should be "the CMA Study (1996)."

vii. Check following references:

a. Abdel-Rahman *et al.* "...C102, C102 and C103" (p. 29)
b. Add space between the first two Suh *et al.* references (p. 32)
c. I could not find "US EPA Guidelines for Developmental Toxicity Risk Assessment" (1996). I searched EPA's web site and used our professional

search group. I did find the Reproductive Toxicity Risk Assessment Guidelines (1996) and the U.S. EPA Guidelines for Developmental Toxicity Risk Assessment, *Federal Register* 56(234), 63798-63826 (December 5, 1991) (p. 33 of Report).

d. Please complete reference, U.S. EPA National Primary Drinking Water Regulations (p. 34).

7. Comments on the Critical Study: CMA (1996) Two-Generation Study of Sodium Chlorite Administered in the Drinking Water to Rats

This reviewer considers the study "adequate"; she has major problems with the design and conduct of this study, as follows:

1. The test material was 81.4% pure. Analysis including identity of the impurities is provided (p. 118), but there is no explicit rationale for the use of a test material with this purity. For example, is this the "typical" profile of water disinfected with sodium chlorite?

2. The level of purity was corrected for by adjusting the concentration of test material in the drinking water by a factor of 1.23 (p. 31). It is hoped that the actual concentrations of test material are listed somewhere in the report (I only have Vol. 1 of 6).

3. Pups selected for neurobehavioral analyses were not exposed to the dosed drinking water after weaning (postnatal day 21) until all the tests were performed, on or about postnatal day 60 (noted in the risk assessment document and "explained" by the CMA response) thereby reducing the opportunity to produce and detect effects.

4. The F2a females were not evaluated for preputial separation. The text of the report indicates that "with the exception of 20 males and 20 female pups retained for evaluation of auditory startle response, the F2a pups were killed on post natal day (PND) 24 and subjected to necropsy." (p. 25). BUT Section 16.0, Deviations from the Protocol, item no. 24 indicates "F2a generation vaginal preforation was not recorded until the majority of the females had already achieved the criteria in error." (p. 183). The F2a females must have been older than pnd 24 for the "majority of females" to achieve vaginal patency since the mean age at acquisition is 33-34 days (CMA response).

5. According to Section 16.0, Deviations from the Protocol, no. 24 (page 25): "For the [F2a] males there was a delay of four days prior to the commencement of observations for preputial separation. However, this was considered to have a minimal effect and hence the data was reported." The report does not specify at what age the F2a male rats began to be evaluated, but this error may have been responsible, at least in part, for the apparent lack of an effect on this parameter in F2a males, *i.e.*, the acquisition in controls may have been earlier than indicated.

6. Section 16.0, Deviations from the Protocol, item no. 17, indicates that although the protocol specifies collection of weight of the coagulating gland, it was not done. The reason as indicated was: "However, as this is part of the seminal vesicles and as such cannot be weighed separately, the weight of the seminal vesicles included that of the coagulating gland." This reason is not acceptable. My laboratory (and many others) separates the coagulating glands from the seminal vesicles routinely for weighing and histopathology. This apparent ignorance of relatively standard procedures in

reproductive toxicity studies causes concern for performance of other aspects of the study.

7. The performing laboratory considered 300 ppm as the NOEL (no observable effect level) for reproductive toxicity of the parents and offspring and for developmental neurotoxic effects and 70 ppm the NOAEL (no observable adverse effect level) for hematologic effects (with the NOEL as at "or closely approximate to" 35 ppm) (page 27, Conclusions). They appear to be discounting their own data without suitable explanation or rationale.

Peer Review of the Draft Health Risk Assessment/Characterization of the Drinking Water Disinfectant Chlorine Dioxide and the Degradation Byproduct Chlorite.

In general, the Draft report is well written and provides a good overview of the chlorine dioxide and chlorite toxicity studies. The key endpoints most relevant to human heath are properly identified as potential reproductive and neurodevelopmental toxicity, based on several lines of evidence. Previous studies are of limited quality for use in risk assessment.

Identification of Critical Effect(s) and Setting a NOAEL

A recent CMA-sponsored 2-generation reproductive and developmental toxicity study is appropriately identified as the critical study for development of a RfD for both chlorine dioxide and chlorite. However, I disagree with the NOAEL of 35 ppm (3 ClO_2^- mg/kg-day) used in this Draft Health Risk Assessment/Characterization document. Considerably more description is needed in the Draft Health Risk Assessment/ Characterization document to justify the selection of the NOAEL and LOAEL used.

The document lists the following significant alterations related to treatment, (listed on page 16):

300 ppm

- reduced liver weight in F0 ♀, and F1 ♂ & ♀
- reduced pup survival (increased number of pups found dead and/or killed during lactation)
- reduced pup body weight at birth and throughout lactation in F1 and F2
- lower thymus weight in F1 and F2
- lower spleen weight in F1 and F2
- lower incidence of pups exhibiting a normal righting
- lower incidence of pups with eyes open on PND 15
- altered clinical condition in F2 animals chosen for neurotoxicity
- decreased pup brain weight
- delays in sexual development in ♂ (preputial separation) and ♀ (vaginal opening) in F1 and F2
- lower RBC parameters.

70 ppm

- reduced liver weight in F0 ♀, and F1♂ and ♀
- reduced pup survival in F1 and F2
- reduced pup bodyweight in F1 and F2
- minor changes in blood cell parameters in F1
- alterations in clinical condition (e.g., pallor and piloerection)
- lowered auditory startle amplitude
- possible alteration in learning

35 ppm

- minor changes in red blood cell parameters

The Draft Health Risk Assessment/Characterization document (page 17) then identifies a *Developmental* NOAEL of 35 and a *developmental* LOAEL of 70 ppm based on:

1. decreased offspring viability
2. decreased offspring body weight
3. lowered auditory startle amplitude
4. possible alterations in learning behavior

The Draft Health Risk Assessment Characterization document identifies a *Systemic* NOAEL of 35 ppm and *systemic* LOAEL of 70 ppm based on:

1. altered organ weights in the dams

However, when one considers each of the above parameters used as a basis for the NOAEL and LOAEL, considerable questions are raised. Each of these parameters is considered below:

Decreased offspring viability:

Results from the CMA-sponsored 2-generation reproductive and developmental toxicity study of 1996 give little indication of dose-dependent decreased offspring viability in the F1 or F2 generations. After reviewing Tables 17, 51, and 60 of the CMA-study report I fail to understand how a NOAEL of 35 ppm and a LOAEL of 70 ppm were derived based on decreased offspring viability. Comparison of the Gestation Indexes for F1, F2a and F2b groups does not support a treatment related effect at 70 ppm (93.1, 100, 100 Gestation Index, for the F1, F2a, and F2b offspring, respectively, that were exposed *in utero* to 70 ppm).

No statistically significant between-group differences were measured in Mean Viability Indexes, Mean Number of Pups Born, or Mean Cumulative Survival Indexes by Analysis of Variance/Kruskal-Wallis analysis. If further statistical analyses were conducted on these data, they were not made available to this reviewer.

The variations from control groups were small. It is not apparent that any small decreases from control were treatment related. Therefore, based on these results, the NOAEL for offspring viability should be 300 (or possibly 70 ppm) rather than 35 ppm.

Decreased offspring body weight:

Review of Tables 19, 53 and 62 of the CMA-study report indicates that statistically significant differences in pup bodyweight at parturition or at various days post-partum only occurred in the highest treatment group (300 ppm). In the F1 generation, a small significantly lower mean birthweight was reported in both male and female pups, and this lower weight persisted throughout 24 days post-partum. No statistically significant differences from control values were reported for the 35 ppm or 70 ppm groups.

In the F2a and F2b generations, only F2a males in the 300 ppm treatment group had significantly lower birthweight than the corresponding control group. No statistically significant differences from control values were reported for the 35 ppm, 70 ppm, or 300 ppm F1a female, F2b male or F2b female groups.

Therefore, significant differences in offspring bodyweight were detected only in the highest treatment group of 300 ppm of either F1 or F2 generations. Subsequently, based on this parameter, the LOAEL should be 300 ppm and the NOAEL should be 70 ppm. It is not clear how the authors selected a NOAEL of 35 ppm using offspring bodyweight as a parameter of adverse effects.

Lowered auditory startle amplitude:

The Draft Health Risk Assessment/Characterization Report states that lowered auditory startle amplitude was measured in chlorite-treated groups. Indeed, the CMA-study report indicates,

> "On day 24 post-partum the maximum startle amplitude tended to be decreased compared to control in most trial blocks for groups 3 and 4. These differences achieved statistical significance when compared with the controls. There were no statistically significant changes observed in the Day 60 post-partum assessments."

However, from the results resented in the CMA-study report there is no convincing evidence that a treatment related, biologically relevant, adverse effect was measured. There are several reasons to reject the acceptance of 35 ppm as the NOAEL based on the auditory startle-habituation response test results:

- Three parameters were measured in the auditory startle-habituation response, time to maximum amplitude of response, maximum amplitude of response, and habituation to response. No differences were measured between control and treated groups for measurements of maximum amplitude of response or habituation of the startle response. Only the maximum startle amplitude was described as statistically significant in the two highest dose groups. The mean data for the auditory startle amplitude response are presented in Table 43, page 1194, of the CMA-study report.

  Review of these results raises several questions.

  1. It is not clear how the authors of the CMA-study report determined that the differences in the two highest doses achieved statistical significance. None of the maximum amplitude values in Table 43 are designated with an asterisk; hence no indications of statistical significance are given in the table.
  2. No consistent dose-dependent decreases were seen between the different trial blocks in either the male or females. For example, when the data are expressed as percent of the control group within each trial block:

Maximum Amplitude of Response to Stimulus: Males, day 24 post-partum

Trials	35 ppm	70 ppm	300 ppm	Comment
2-10	0.95	0.85	0.85	
11-20	0.84	0.84	0.82	Any real significant differences between doses?
21-30	0.85	0.88	0.87	No real significant differences between doses? Lowest exposure group has greatest decrease in maximum amplitude.
31-40	0.83	0.90	0.86	Lowest exposure group has greatest decrease in maximum amplitude.
41-50	0.89	0.89	0.91	No dose-dependent effect. Any real significant differences between doses?

Maximum Amplitude of Response to Stimulus: Females, day 24 post-partum

Trials	35 ppm	70 ppm	300 ppm	Comment
2-10	1.14	0.92	0.96	No consistent dose-dependent decrease. Mid-exposure group has greatest decrease in maximum amplitude.
11-20	1.19	0.77	0.95	Any real significant differences Control and high dose? Mid-exposure group has greatest decrease in maximum amplitude.
21-30	0.98	0.74	0.80	No consistent dose-dependent decrease. Mid-exposure group has greatest decrease in maximum amplitude.
31-40	0.91	0.72	0.72	No consistent dose-dependent decrease.
41-50	0.94	0.68	0.70	No consistent dose-dependent decrease. Mid-exposure group has greatest decrease in maximum amplitude.

These results are very inconsistent. In none of the trial blocks does maximum amplitude decrease with increasing dose. Within three trial blocks a decreased maximum amplitude in the two highest treatment groups suggest a possible threshold effect between 35 ppm and 70 ppm (Trials 2-10 in males and trials 31-40 and 41-50 in females), but this is so inconsistent that no conclusions can be made. Therefore, these results are too inconsistent and the interpretation too equivocal to base any NOAEL or RfD value.

- The effect on maximum startle amplitude was transitory (if there really was an effect). Maximum startle amplitude responses at 60 days post-partum were not significantly different.
- No histopathological evidence was detected to support a conclusion of developmental neurotoxicity.

Therefore, interpretation of the auditory startle-habituation response test results as a measure of developmental neurotoxicity seems equivocal, at best. Unless further analysis and discussion are provided, I do not accept the auditory startle amplitude data as a basis for setting a NOAEL at 35 ppm.

Possible alterations in learning behavior:

After reviewing Tables 24-31 of the CMA-study report, I fail to find evidence for a NOAEL of 35 ppm based on "possible" alteration in learning behavior.

Likewise, any small decrease in absolute brain wet weights are more likely a function of smaller body weights rather than a direct treatment related effect to the CNS. No significant differences were detected in brain weights when the data are expressed as a ratio of organ weight to body weight.

Therefore, I reject the NOAEL of 35 ppm for the chlorite 2-generation reproductive/developmental toxicity study of 1996. The LOAEL for this study should be

300 ppm and the NOAEL for this study should be 70 ppm (5.9 ClO_2^- mg/kg/day, rounded to 6 ClO_2^- mg/kg/day).

An argument can still be made that the effects measured in treated rats of the CMA-study were most likely due to decreased food and water intake caused by altered palatability of the chlorite-containing water supply. As pointed out by one of the original peer reviewers of the CMA-study (and it was not me), it is quite possible that the true NOAELs from direct chemical exposure, other than palatability, are higher than indicated by the data. While this possibility may add some degree of uncertainty to the risk assessment of chorite, it errors on the side of caution. Therefore, a NOAEL of 70 ppm is a prudent, protective value, since the true value likely is higher.

Calculation of Oral RfD

The approach for calculating the oral RfD for chlorite is valid and appropriate. An uncertainty factor of 100 is appropriate for calculating the RfD from the animal study. The additional factor of 3 is no longer needed to account for the lack of a multigenerational reproductive study. While the approach is appropriate, the number for the NOAEL needs to be revised to 6 ClO_2^- mg/kg-day, as recommended above, resulting in an oral RfD of 0.06 mg/kg-day.

Other comments:

The identification of the last two potentially susceptible populations on page 25 seems highly speculative. While it is true that G-6-PDH-deficient individuals and NADH-dependent methemoglobin reductase-deficient individuals may be more susceptible to oxidative agents, it is not at all clear that this is the mechanism by which chlorite exerts any reproductive or neurodevelopmental toxicity. The identification of these sensitive populations is based on supposed mechanistic insights. The potential mechanisms discussed on page 23 are hypotheses. Subsequently, the identification of these populations as potentially more susceptible to reproductive or developmental effects of chlorite seems to be speculation based on speculation. The level of confidence for identifying these subpopulations as uniquely susceptible is very low.

Specific comments – suggested editorial revision

Page 5, first full paragraph, next to last sentence: "The quality of the untreated well ...it contained and chemical..." Change "and" to "any".

Page 5 first full paragraph, last sentence: "The atypical baseline data raise concerns about control..." Insert "the" after "about".

Table 2, page 8, last column for the Haag (1949) reference. I suggest you indicate that the authors attributed the renal effects to a nonspecific salt effect.

Page 15, line 2. "What malformations fell..." Change "What" to Which". Better yet, revise the sentence to read, "The authors did not state which malformations fell into each of these categories."

Page 15, last paragraph: "Males were exposed throughout mating then..." Insert "and" after "mating".

Page 15, last full sentence: "Water concentrations of sodium chlorite..." Revise to read, "Sodium chlorite concentrations were adjusted downward..."

Page 23, third full paragraph, first line: Correct "Harringon" to "Harrington".

Overall Summary:

The overall report is acceptable and adequate for risk assessment for chlorine dioxide and chlorite. My only major disagreement is with the choice of the NOAEL used to calculate the RfD, as described above.

Comments on

Draft Health Risk Assessment/ Characterization Of The Drinking Water Disinfectant Chlorine Dioxide And The Degradation Byproduct Chlorite

General Comments

Overall I found this a reasonably balanced report based on the supporting materials provided. I believe that the study chosen as being critical (the CMA 2-generation reproduction study) was the correct study to select for risk assessment purposes, based on the changes noted and the overall quality and age of the study.

It is also this reviewer's understanding that a complete review of the critical study is NOT requested, only a review of the risk assessment document.. Therefore, I have only used the report to clarify certain statements and have not examined the reviews for the scientific critique of the report itself.

The comments that I have do not change the approach, study selection or overall RfD produced. They are more matters of clarification and where more detail could be provided to sustain and improve the case being proposed.

Specific comments

1. Introduction.

The statement is made (p3, para 2) regarding the dissociation of chlorine dioxide under aqueous conditions and in the GI tract of animals. Can the reviewers provide

references to sustain this view. It is important since in the last sentence of the paragraph the key point is made that the toxicity data for one compound are considered applicable for addressing toxicity data gaps for others. This is a major assumption and should be qualified to the maximum extent possible.

2. Hazard assessment

The statement (p4, 2nd line) that carcinogenicity data from animal studies are largely inadequate could be qualified. (See later comments).

3. Studies in humans

I would like to see which human data are available on hematological parameters. That these agents can cause methemoglobin etc. seems to be well understood but does not feature in this section - it should.

4. Studies in animals - systemic toxicity.

Nasal lesions and potential confounding with direct exposure to drinking water (p6, 4th para). This section could be expanded a little to explain why this potential artifact of treatment was noted. There are examples of chemicals that produce nasal lesion via true oral exposure. I would imagine that a fuller description of the nasal lesion could convince the reader whether inadvertent treatment to the nasal passages was likely to happen (e.g. how deep into the turbinates was the lesion detected etc.) This is an important point if these nasal observations are to be placed in the appropriate perspective for potential risk assessment.

Table 1. (p7)

Could some addition be made to the end points section as to whether the observations are considered to be adverse effects (e.g. hematological changes etc.). A laundry list is not helpful and some simple clarification would help.

5. Reproductive and developmental studies

Harrington study. (P11, 5th para)

Was water consumption presented? There is a tremendous physiological demand on the pregnant dam to drink fluids, whether the water is palatable or not. A presentation of water consumption data, or summary statement would help.

Why in the 26 and 40 mg/kg/d groups does the finding of significant growth retardation (- decreased fetal weights, incomplete fetal ossification), not considered an adverse event ? The developmental risk assessment guidelines clearly state that an alteration of growth, even in the presence of maternal toxicity, should be considered adverse, unless evidence is presented to satisfy that the events were due to a secondary (maternal) event. This reviewer would argue that the growth retardation should not be excluded, particularly if it was consistent and dose -related and would therefore affect the assignment of a developmental NOAEL for the study.

CMA study (p15, 3rd para)

I am perplexed by the statement (p17 2nd para and p26 3rd para) - "Discontinuation of exposure for the animals undergoing neurotoxicity testing minimizes the likelihood of finding a positive effect." And precludes comparison etc. The developmental neurotoxicity testing guidelines indicate exposure should be from gestation day 6 until postnatal day (pnd) 10 with examination of offspring at a number of times post exposure up to pnd 60. Moreover, the guidelines state that that using offspring from a multigeneration study is acceptable. Therefore, the study meets all of the requirements for a developmental neurotoxicity study and should be used

appropriately and can be compared to other tests using exactly the same dosing regime, including stand alone studies. I understand from the accompanying material that positive control data were provided to enable such a comparison to be made.

6. Carcinogenicity

The initial statement indicates that there are no long term oral bioassays (p18, 1st para), but in the next paragraph indicate a mouse study over 85 weeks. Why is this not considered adequate ? 18 month studies in mice are normally considered to be of utility in the assessment of carcinogenic potential in this species. These data do not appear to be have taken into consideration (and there may be flaws with this study that this reviewer cannot ascertain) but to make such bold statements appears inappropriate, else more qualification is required of the studies presented.

7. Hazard assessment issues

p24, 3rd para animal studies.. A comment is made "While exposure [to rats and rabbits] was by the same route to the same dosage range for essentially the **same days of gestation**.....(my emphasis). It is essential that the reviewers understand that the period of exposure is the same developmental period (i.e. organogenesis). In these types of studies, it is often the case that the exposure period (during critical windows of development) is of equal importance to the administered dose level.

Based on the comments above on the neurotoxicity evaluation does this increase the reviewers confidence in the CMA study from medium upwards ?

8. Oral reference dose (p27, section 3.2)

Did the reviewers consider the whole reproductive and developmental database to be adequate for Risk Assessment purposes ? Should additional factors be invoked for "child health issues" ? This reviewer believes that these are adequate and that a

further uncertainty factor is not required. However this should be explicitly stated in the report to avoid any confusion.