Toxicology profiles

In the following, the toxicological status of those impurities that are not GRAS or regulated in the CFR is detailed.

1 Sodium borate

1.1 Summary and overall conclusion

In dilute aqueous solutions at physiological pH, sodium borate is predominantly present as boric acid (Moore 1996). Therefore when assessing the toxicity and the potential risk with exposure of minute amounts of sodium borate it is relevant to consider toxicity data from both sodium borate and boric acid.

The major relevant toxicity information on sodium borate/boric acid stems from the National Toxicology Program studies on boric acid. The NTP have conducted genetic toxicity testing, a full program of reproduction and developmental studies, repeat dose toxicity studies in mice and a carcinogenicity study in the same species. The other important data set comes from the publication of Weir and Fisher (Weir and Fisher 1972) where information on repeat dose toxicity is available for rats and dogs (both chemicals) and a carcinogenicity study in rats (also both compounds). This comprehensive data set with many high quality studies facilitate an adequate risk assessment of low dose exposure to sodium borate.

Boric acid/sodium borate was shown to have no genotoxic activity in a relevant set of in vitro genotoxicity studies. No carcinogenic activity was observed in the two life-long studies conducted in mice and rats. It can therefore be concluded that sodium borate confers no carcinogenic risk.

Repeat dose toxicity studies, the two carcinogenicity studies as well as the study on general reproductive performance in mice showed testis to be the most sensitive target organ. Reduced sperm count as well as tubular atrophy was observed at dose level from 150 mg/kg and above. Tubular atrophy was observed in mice, rats and dogs but the NOEL's differed between species being in the range of 25-100 mg/kg. This difference in NOEL could well reflect the large spacing/interval of the doses in the various studies rather than a true difference in sensitivity or target organ exposure.

Developmental toxicity studies in rats and rabbits showed clear evidence of toxicity to the embryo/foetus. Malformation was also observed. The NOEL for developmental toxicity was determined to125 mg/kg based on embryo/foetal deaths and cardiovascular malformations in rabbits.

By using the FDA recommended safety factor of 1000, an ADI value of 25 μg/kg body weight per day is obtained. This value should be compared to the estimated daily exposure to sodium borate from the use of the FCS i.e. 0.0046 μg/kg bw/day. It can therefore be safely concluded that the potential exposure to sodium borate from the use of the present FCS should be of no concern with respect to human health.

1.2 Study summaries

1.2.1 Genetic toxicity studies

Gene mutations in bacteria: Boric acid was tested in the Ames Salmonella assay using the preincubation protocol with tester strains TA98, TA 100, TA 1535 and TA1537 with and without a metabolic activation system (NTP TR 324, 1987). Concentrations of up to 1820 µg/plate was used. No mutagenic activity was observed in the investigated tester strains both with and without a metabolic activation system.

Sodium borate was tested also tested in the Ames Salmonella assay tester strains TA 98 and TA 100 using the preincubation method (Benson et al 1984). No mutagenic activity was observed with and without a rat S-9 metabolic activation system.

Genetic toxicity in mouse L5178Y/TK lymphoma cells: Boric acid was tested in the mouse lymphoma assay with and without a metabolic activation system (NTP TR 324, 1987). Incubations with boric acid were performed for 4 hours whereafter the cells without the chemical were incubated for another 48 hours to allow expression of the mutant phenotype. Concentrations up to 5000 µg/mL were investigated but no overt cytotoxicity was observed at this concentration. Boric acid showed no increase in mutation frequency (mutants/10^6 clonable cells) with and without a metabolic activation system.

Chromosomal aberrations and sister chromatid exchanges in CHO cells: Boric acid was tested for induction of chromosomal aberrations and sister chromatid exchanges in Chinese Hamster Ovary (CHO) cells both with and without a metabolic activation system (NTP TR 324, 1987). The highest concentration investigated was 2000 µg/mL in the test for chromosomal aberrations and 500 µg/mL in the test for SCE's. No increase in chromosomal aberrations or SCE's was observed in the presence or absence of a metabolic activation system.

Overall conclusion on the genotoxicity of boric acid/sodium borate

Boric acid have been investigated in a relevant set of in vitro tests for genotoxic activity (Ames Salmonelly assay, Mouse Lymphoma and in a vitro assay for chromosomal aberrations and SCE's in CHO cells) without any evidence of a genotoxic effect. Sodium borate that once in solution at physiological pH should primarily (approx. 98%) consist of un-dissociated boric acid also showed no evidence for mutagenic activity when tested in the Ames Salmonella assay; two tester strains only. It can thus be concluded that sodium borate has no mutagenic/genotoxic activity.

1.2.2 In vivo toxicity tests

Repeat dose toxicity studies

Thirteen-week repeat rose study in mice: Groups of 10 male and 10 female mice were fed boric acid at concentrations of 0, 1,200, 2,500, 5,000, 10,000 and 20,000 ppm for thirteen weeks (NTP TR 324, 1987). Eight male mice and 1 female mouse receiving 20,000 ppm and 1 male receiving 10,000 ppm boric acid died before the end of the studies. Male and female mice receiving 20,000 ppm boric acid weighed 23% and 18% less, respectively, than did the controls at the end of the studies. Testicular atrophy in 8/10 male mice, hyperkeratosis and acanthosis of the stomach in 8/10 male and female mice, and extramedullary hematopoiesis of the spleen in all male and female mice receiving 20,000 ppm boric acid indicated that the testis, stomach, and spleen were potential target organs in the 2-year studies. Based on these results, 2-year NTP toxicology and carcinogenesis studies were conducted by feeding diets containing boric acid at concentrations of 0, 2,500, or 5,000 ppm to groups of 50 male and 50 female mice.

90-day repeat dose study in rats: A diet of borax or boric acid was administered to Sprague Dawley rats (10/sex/dose) for 90 days at doses of 0, 52.5, 175, 525, 1,750 or 5,250 ppm as boron equivalents (Weir and Fisher 1972). Both borax and boric acid produced 100% mortality at the highest dose and complete atrophy of the testes in all males fed diets containing 1,750 ppm boron equivalent. At 1,750 ppm boron, both compounds produced significant decreases in body weight and in the mean weights of the liver, kidney, spleen, and testes. At lower doses, changes in organ weights were inconsistent. At 52.5 ppm boron, borax produced increases in the mean weights of the spleen, kidneys, and ovaries in females, and boric acid produced an increase in liver mean weight. These changes were not observed at 525 ppm boron for either compound. Microscopic examination revealed partial testicular atrophy at 525 ppm boron in four males fed borax and in one male fed boric acid.

90- day repeat dose study in dogs: A diet containing 0, 17.5, 175, or 1,750 ppm boron in the form of borax or boric acid was administered to groups of five male and five female beagle dogs for 90 days (Weir and Fisher 1972). Except for one death in a male dog at the 1,750 ppm boron level (as borax), dogs fed both boron compounds were normal in appearance, behavior, elimination, body weights, and food consumption. At 1,750 ppm, both compounds produced significant ($p< 0.05$) decreases in thyroid and testes to body weight ratios and severe testicular atrophy with degeneration of the spermatogenic epithelium in all male dogs. At 175 ppm boron (as boric acid), a decrease in testes to body weight ratio was observed. This effect was not accompanied by histological changes.

Reproduction toxicity studies

Reproductive toxicity of boric acid in mice*:* The potential reproductive toxicity of boric acid (BA) in CD- l (Swiss) mice was evaluated using the Reproductive Assessment by Continuous Breeding (RACB) Protocol (NTP # RACB88034, 1990). Male and female mice were exposed to BA at concentrations of 0, 1000, 4500, or 9000 ppm in the feed; this produced estimated consumption levels of approximately 152, 636, and 1262 mg/kg body weight.

During 14 weeks of cohabitation with continuous access to a BA-containing diet, no litters of dead or live pups were produced by 9000 ppm cohabited pairs. Among the litters born to pairs fed BA at 4500 ppm, live litter size and pup body weight were significantly reduced in comparison to controls. All aspects of fertility were unaffected at 1000 ppm BA.

A crossover mating trial (Task 3) at the end of the continuous cohabitation phase, using the middle dose group, confirmed the male as the affected sex, with observed fertility rates of: 0 ppm male x 0 ppm female, 74%; 4500 ppm male x 0 ppm female, 5%; and 0 ppm male x 4500 ppm female, 65%. The mating index was 79%, 30%. and 70% for the same groups. Additionally, adjusted body weights, for pups born from the mating of control male x 4500 ppm female, were significantly decreased from controls ($P < 0.01$), indicating that the female and/or pup are also affected by BA.

At sacrifice, after 27 weeks of BA exposure, the F_0 males fed 9000 ppm BA had significantly lower body weight and reproductive organ weights (testes, combined caput and corpus epididymis. and cauda epididymis) and significantly fewer spermatozoa in the cauda epididymis. Males fed 4500 ppm BA also had significantly lower testes, epididymis, prostate weight. and fewer spermatozoa in the cauda epididymis. Organ weights were unaffected at 1000 ppm BA for the F_0 males.

The germinal epithelium of F_0 males in the 9000 ppm group was atrophied and consisted mostly of Sertoli cells with occasional spermatogonia. The 4500 ppm group had fewer spermatids than in the controls; multinucleate giant cells were observed.

Sperm concentration per mg cauda was dramatically reduced in 9000 ppm males (2.8±1.7 x 10^3) compared to controls (519±36 x 10^3). Motility was difficult to quantify due to extremely low sperm concentrations. In 4500 ppm males, both sperm concentration (146.9±26.5 x 10^3) and sperm motility (53.3± 8.2%) were lower than in controls (519±36 x 10^3 sperm with 78.1±3.0% motility). Males fed 1000 ppm BA had normal sperm concentrations with reduced motility (69.0±4.5%).

At necropsy, F_0 female body weight was significantly decreased in the high dose group. The F_0 females in the 4500 ppm group had significantly decreased kidney/adrenals and liver weights.

The F_1 mice exposed to dietary BA (0 and 1000 ppm), beginning at conception, had normal fertility. The adjusted mean body weight of F_2 pups was decreased. However. the number of live pups per litter, the proportion of pups born alive, sex of pups born alive, and unadjusted weights of pups born alive, were not significantly changed by BA exposure. At necropsy, F_1 males had normal reproductive organ weights and sperm motility. However. BA treatment decreased sperm concentrations in F_1 males (585.6±32.5 x 10^3 in controls vs. 442.6±51.2 x 10^3). Female F_1 mice had significantly greater uterus and kidney/adrenal weights than controls.

This study confirms that BA is a reproductive toxicant in mice, primarily through an effect in the male. The 1000 ppm dose approached a No Observed Adverse Effects Level (NOEL) for the adult reproductive system, as well as for the developing reproductive system.

Developmental toxicity of boric acid in mice*:* Boric acid (BA) was tested for developmental toxicity in timed-mated CD-1® mice (NTP #TER89028, 1989). BA (0, 0.1, 0.2 or 0.4% in feed) was administered from gestational day 0 to 17 with average intakes of 248, 452 or 1003 mg/kg/day.

Dams exposed to 0.4% BA exhibited decreased weight gain during treatment (gestation) compared to controls, even though food and water consumption were not reduced. Gestational weight gain corrected for gravid uterine weight was not affected. High-dose BA caused increased water consumption during late gestation (gd 15-17) and increased relative kidney weight. A dose-related incidence of renal tubule dilatation/regeneration was observed in 0/10, 2/10, 8/10 and 10/10 randomly selected dams in the control through high-dose groups.

Reduction of fetal body weight was dose-dependent (94%, 89% and 66% of controls), but statistically significant only at 0.2 and 0.4% BA. The high-dose group had an increased percentage of resorptions (19% vs. 6% for controls) and malformed fetuses/litter (9% vs. 3% for controls). The most apparent treatment-related morphological changes involved deficient rib development at the thoracic-lumbar junction, i.e., an increased incidence of short rib XIII (a malformation) and a decreased incidence of rudimentary or full rib(s) at Lumbar I (an anatomical variation).

In summary, maternal renal toxicity was observed at all BA exposures. The low exposure (0.1%) approached the maternal no-observed adverse effect level (NOEL) with mild renal lesions in only 2 of 10 females. The NOEL for developmental toxicity of BA was 0.1%.

Developmental toxicity of boric acid in rats: Boric acid (BA) was administered in the feed at concentrations of 0.1%, 0.2% or 0.4% on gestational days (gd) 0 to 20 (n=29/group) to timed-mated Sprague-Dawley rats (NTP # TER89027, 1990). Exposure to 0.8% dietary boric acid (n=14) was restricted to the period of major organogenesis (gd 6 to 15) in order to limit early embryolethality.

Average daily intake of boric acid was 78, 163, 330 and 539 mg/kg/day , respectively. Exposure to 0.2% and 0.4% resulted in increased maternal food intake for gd 12 to 20; water intake was increased on gd 18, to 20 at 0.4%. Food intake was decreased during treatment at 0.8% with a rebound increase on gd 15 to 18; water intake was decreased on gd 6 to 9. Other effects summarized across all dose levels included increased relative maternal liver and kidney wts. at

greater than or equal to 0.2%, decreased gravid uterine weight at greater than or equal to 0.4%, decreased wt. gain during treatment and gestation at greater than or equal to 0.4%, and increased corrected body wt. gain only at 0.4%. Microscopic evaluation of maternal kidneys (10 dams/group) did not provide any definitive evidence for treatment-related renal pathology.

Average fetal body wt./litter was reduced at all doses. Prenatal mortality was increased only at 0.8%. The incidence of fetal malformations was significantly increased at greater than or equal to 0.2% dietary boric acid (2, 3, 8, 50 and 73% malformed fetuses/litter in the control through high-dose groups). The most frequently observed malformations were enlarged lateral ventricles of the brain, and agenesis or shortening of rib XIII. As an associated finding, the incidence of Lumbar I rib(s), a common variation in the CD® rat, was reduced following boric acid treatment.

In conclusion, the NOEL for maternal toxicity was 0.1% dietary boric acid and the LOEL was 0.2%. Embryo/fetal toxicity occurred in all treatment groups (greater than or equal to 0.1%).

Developmental toxicity of boric acid in rabbits: Artificially-inseminated New Zealand White (NZW) rabbits (30 per group) were exposed to 0, 62.5, 125 or 250 mg/kg/day of boric acid (NTP #TER90003, 1991). Aqueous solutions were delivered by gavage in a volume of 5 ml/kg on gestational days (gd) 6-19.

Pregnant does exhibited no overt symptoms attributable to boric acid toxicity, except that vaginal bleeding was noted at 250 mg/kg/day (2-11 does/day on gd 19-30). All high-dose does with this symptom had no live fetuses on gd 30. Vaginal bleeding was not observed in any control females, and in only one female/group at 62.5 or 125 mg/kg/day (day 20 or 22, respectively; each female had 5-7 live fetuses at term). Maternal deaths (one each in the 62.5 or 125 mg/kg/day groups on gd 25 and 22, respectively) were not clearly related to boric acid treatment.

Maternal food consumption was decreased during most of the treatment period (gd 6-15) at 250 mg/kg/day, and was increased at 125 and 250 mg/kg/day after treatment (gd 25-30). Maternal body weight (gd 9-30), weight gain during treatment (gd 6-19), gravid uterine weight and number of corpora lutea per dam were each decreased at 250 mg/kg/day. Corrected maternal weight gain was increased at both 125 and 250 mg/kg/day . Maternal liver weight (absolute or relative) was not affected by boric acid exposure. Relative maternal kidney weight was increased at 250 mg/kg/day , but absolute kidney weight was not affected. Microscopic evaluation of maternal kidney sections did not indicate any pathology associated with boric acid exposure. Thus, 250 mg/kg/day was the LOEL for pregnant does, and 125 mg/kg/day was the maternal NOEL.

No definitive evidence of developmental toxicity was observed following exposure of pregnant does to either 62.5 or 125 mg/kg/day boric acid during the period of major organogenesis (gd 6-19). At 250 mg/kg/day , developmental toxicity included a high rate of prenatal mortality (90% of implants/litter were resorbed vs. 6% for controls). Prenatal mortality was also expressed as an increased proportion of pregnant females with no live fetuses (73% vs 0% of controls) and as a reduction in the number of live fetuses/live litter on gd 30 (2.3/litter vs. 8.8 for controls). The incidence of malformed live fetuses/litter was also increased at 250 mg/kg/day (81% vs. 26% for controls), primarily due to the incidence of fetuses with cardiovascular defects (72% vs. 3% for controls). The most prevalent cardiovascular malformation was interventricular septal defect which was observed in 57% (8/14) of high dose fetuses as compared to 0.6% (1/159) of fetuses in the control group. At 250 mg/kg/day , the average fetal body weight/litter was 92% of the average control weight, but this difference did not reach statistical significance. Thus, 250 mg/kg/day was the LOEL for developmental toxicity and 125 mg/kg/day was the NOEL.

In summary, decreased food intake and vaginal bleeding associated with pregnancy loss were the only clear manifestations of toxicity in does exposed to 250 mg/kg/day boric acid on gd 6-19. The same dose was associated with severe developmental toxicity, i.e. 90% prenatal

mortality/litter and 81% malformed fetuses/litter. No definitive maternal or developmental effects were observed at 62.5 or 125 mg/kg/day.

CNS developmental toxicity of boric acid in rats exposed on gd 14-17: In an earlier study, exposure of boric acid (BA) in rats during gd 6-15 resulted in an increased incidence of enlarged lateral ventricles (ELV). These findings were accompanied by severe reductions in fetal body weights (63% and 46% of control weights, respectively). The present study was conducted to determine whether induction of ELV could be separated from fetal body weight deficits by focusing the exposure around a known sensitive period for induction of hydrocephaly in rat fetuses. The potential contribution of decreased maternal food intake to the developmental toxicity at 2.4% boric acid was also examined.

BA (0, 0.8, 1.6 or 2.4% in the diet) was provided to timed-mated female rats from gd 14 to 17 (NTP # TER90123, 1994). During the treatment period, the vehicle control group was fed undosed diet ad libitum, and a pair-fed control group was provided with the median amount of food consumed (g of food/kg of body wt/day) by the 2.4% BA group. During the remainder of the study, dams and pups had ad libitum access to undosed diet, except during the second week of lactation (Phase I) when food consumption exceeded the available supply of food during one measurement period. Thus, dam and pup data collected on pnd 14, 21 and 26 may have been compromised due to this temporary shortage of food.

The pair-fed control group did not differ significantly from the ad libitum vehicle control group for any parameter related to offspring development in either phase of this study. In contrast, adverse effects were observed in all BA-exposed groups. Fetal body weight was significantly reduced in the low, mid, and high dose BA groups on gd 20 (82%, 68% and 69% of control weight, respectively). By pnd 21, recovery from body weight deficits was complete for the low dose group (104% of control weight). Persistent body weight deficits were observed at the mid dose (83% of control weight; biologically relevant but not statistically significant) and at the high dose (75% of control weight: statistically significantly).

Prenatal mortality was not affected on gd 20 (Phase II), but cumulative post-implantation mortality through pnd 21 was increased at the high dose (34%, 44%, 33% and 72% for the vehicle control through high-dose BA groups, respectively). The incidence of treatment-related mortality was most pronounced during the early postnatal period (pnd 0 to 4) as follows: 2%, 8%, 27% and 44% of pups per litter in the vehicle control through high-dose BA groups, respectively.

No treatment-related effects were noted for the incidence of craniofacial, palatal or CNS structural defects. Changes in absolute brain weights (regional or total) observed in the mid- and high-dose groups were proportional to alterations in body weight on pnd-26, except that the relative weight of the medulla/pons was increased at the high dose. Analysis of brain regions (telencephalon, diencephalon, medulla oblongata/pons and cerebellum) as a percentage of total brain weight indicated that the relative weight of the telencephalon was decreased, while the relative weight of the medulla/pons was increased. No effects on regional or total brain weights were observed at 0.8% BA.

In summary, gd 14 to 17 was not a sensitive period for BA-induced ventricular enlargement. However, fetal body weight was reduced at all doses. Low-dose offspring recovered completely from growth deficits by pnd 21, but body weight effects persisted in the mid- and high-dose groups. These results indicate that BA can reduce fetal body weight to 69% of control weight on gd 20 without a concomitant increase in the incidence of ventricular enlargement.

CNS developmental study of boric acid in rats exposed on gd days 6-15: A previous standard developmental study in Sprague-Dawley rats showed enlarged ventricles of the brain (ELV's) (NTP # TER89027, 1990). This effect could not be repeated in "window-treated (gd 14-17) animals (NTP # TER90123, 1994). This study was therefore designed to determine (1)

whether the induction of ventricular enlargement could be experimentally repeated following exposure to BA on gd 6 to 15, (2) whether the incidence would exhibit a dose-response relationship at exposure concentrations of 0.4% to 0.8% BA in the diet, and (3) whether the incidence and severity of ventricular enlargement would change during postnatal life.

Thus, BA (0.4%, 0.5%, 0.6% or 0.8% in the diet) was provided to timed-mated CD® rats (42-76/group) from gd 6 to 15 (NTP #TER93138, 1994). In Phase I (teratology evaluation), dams were terminated and the uterine contents evaluated on gd 20. In Phase II, dams were allowed to deliver and rear their litters until postnatal day (pnd) 21. In both phases, offspring were evaluated for post implantation mortality, body weight and morphological development of the head (gross morphology of external and internal structures). Phase I (and Phase II) dams ingested average doses of 299 (283), 361 (368), 432 (434), or 549 (562) mg BA/kg/day in the low- through high-dose groups, respectively.

Exposure to 0.4-0.8% BA was associated with dose-related intrauterine growth retardation. Complete recovery from growth deficits was observed prior to the end of lactation at 0.4-0.6% BA, but not at 0.8% BA. The LOEL for post implantation mortality was 0.4% BA. After adjusting for fetal body weight by covariate analysis, the incidence of ELV showed no significant dose-response relationship, but the incidence of hydrocephaly was increased at all doses. These results support the interpretation that BA exposure during organogenesis adversely affects CNS development in the rat independent of its effect upon fetal growth. The LOEL for adverse CNS effects in this study was 0.4% BA in the diet, consistent with the outcome of the prior investigation in which CNS findings were observed at 0.4% (gd 0 to 20) and 0.8% (gd 6 to 15), but not at less than or equal to 0.2% BA (gd 0 to 20).

Carcinogenicity studies

Carcinogenicity study in rats. Groups of 35 Sprague Dawley rats/sex were administered dietary concentrations of 0, 117, 350 and 1170 ppm boron equivalents (boric acid and sodium borate) (approximately 0, 67, 200 and 669 mg/kg bw/day of boric acid) for 2 years (Weir and Fisher, 1972).

At the highest dose groups, both boron compounds lowered food consumption during the first 13 weeks of study and suppressed growth throughout the study. Testes weights and testes to body weight ration were significantly ($p<0.05$) decreased, and brain and thyroid to body weight ratios were significantly ($p<0.05$) decreased. The seminiferous epithelium was atrophied, and the tubular size in the testes was decreased. No treatment related effects were observed in rats treated with either 350 or 117 ppm boron as sodium borate or boric acid.

Thus, this study showed no evidence of carcinogenic activity of sodium borate or boric acid in male or female Sprague-Dawley rats.

Carcinogenicity study in mice. Groups of 50 B6C3F1 mice/sex were administered boric acid in the diet at concentrations of 0, 2,500, or 5,000 ppm for 2 years (NTP TR 324, 1987).

Survival of high dose male mice after week 63 and of low dose mice after week 84 was lower than that of the controls (final survival: control, 41; low dose, 30; high dose, 22), which may have reduced the sensitivity of the carcinogenicity study; the numbers of female mice (33; 33; 37) that survived to the end of the studies were considered adequate for toxicologic evaluation. Body weight gain was reduced in each sex after week 30; mean final body weights were 7% and 13% below control values for exposed male mice and 7% and 20% below those of controls for exposed female mice. No chemically related clinical signs were reported.

With regard to non-neoplastic lesions, boric aced caused at the top dose an increased incidence of testicular atrophy (control, 3/49; low dose, 6/50; high dose, 27/47) and interstitial cell hyperplasia (0/49; 0/50; 7/47) in male mice. The testicular atrophy was characterized by variable

loss of spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa from the seminiferous tubules. The seminiferous tubules contained primarily Sertoli cells and variable numbers of spermatogonia. In some mice, there were accumulations of interstitial cells, indicating hyperplasia.

In low dose male mice, there were increased incidences of hepatocellular carcinomas (5/50; 12/50; 8/49) and hepatocellular adenomas or carcinomas (combined) (14/50; 19/50; 15/49) and an increased incidence of subcutaneous tissue fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) (2/50; 10/50; 2/50). No increased incidence of subcutaneous tissue neoplasms was seen in male mice receiving 5,000 ppm. Because the incidence of subcutaneous tissue tumors is variable in historical controls, because there was no corresponding increase in the high dose male mice, and because the incidence of hepatocellular tumors was not significant by the incidental tumor test and was within the historical control range, neither of these tumors was considered to be related to the administration of boric acid.

Thus, this study showed no evidence of carcinogenic activity of boric acid in male or female B6C3F1 mice.

1.3 NOEL's

Table 1.1 depicts the lowest NOEL's relating to non-neoplastic findings from the repeat dose toxicity, reproduction toxicity and carcinogenicity studies on sodium borate and/or boric acid.

Table 1.1. NOEL's of non-neoplastic findings of sodium borate and/or boric acid

Study type and species	NOEL (mg/kg bw/day)	Effect
90-day repeat dose tox; rat, sodium borate and BA	Approx 50	Testicular atrophy
90-day repeat dose tox; dog, sodium borate and BA	Approx 25	Testicular atrophy
Carc study; Rat, sod. borate and BA	Approx 100	Testicular atrophy
Developmental study; rabbits, BA	125	Litter loss, malformations

The principal target organ and that which is affected at the lowest exposure to boric acid/sodium borate is the testis. The available information clearly shows that the effect is time and dose-dependent. The obtained NOEL'differ somewhat between species but this could well be an effect of the spacing of the dose levels in the various studies. The earliest signs of toxicity decrease in sperm count and focal atrophy (histological observation) are seen at dose levels of 25-100 mg/kg of both boric acid and sodium borate. The similar toxicity for the two compounds is not unexpected knowning that sodium borate is rapidly converts into boric acid once present in a solution at physiological pH.

Rabbit has been shown to be the most sensitive species for embryo-fetal toxicit (litter loss and malformations) with a NOEL of 125 mg/kg.

The NOEL's with regard the principal toxicities identified in the summarized repeat dose toxicity, carcinogenicity and reproduction toxicity studies with boric acid and/or sodium borate are given in Table 2.2 below.

Table 2.2. NOEL's of principal effects identified in toxicity studies with sodium borate and/ or boric acid.

Study type and species	Study ID	NOEL (mg/kg/d)	Effect
13-week toxicity study in mice	NTP TR 324, 1987	1200	Testicular atrophy, hyperkeratosis and acanthosis of the stomach
90 day toxicity study in rats (BA and sodium borate)	Weir and Fisher 1972	60 (BA) 49 (sod bor)	Focal testicular atrophy (LOEL 180 mg/kg BA and 146 mg/kg sodium borate)
90 day toxicity study in dogs (BA and sodium borate)	Weir and Fisher 1972	25 (BA) 20 (sod bor)	Testicular atrophy
2-year car study in mice with BA	NTP TR 324, 1987	300	Testicular atrophy
2-year car study in rats	Weir and Fisher, 1972	120 BA 98 (sod bor)	Testicular atrophy
Reproductive toxicity of BA in mice	NTP # RACB88034, 1990	152	Fertility, reproductive capacity
Reproductive toxicity of BA in mice (27 weeks exp. duration)	NTP # RACB88034, 1990	< 152	Sperm motility
Developmental toxicity of BA in mice	*NTP#* *TER89028, 1989*	242	Foetal body weight
Developmental toxicity of BA in rats	NTP # TER89027, 1990	<163	Average foetal body weight/litter
Developmental toxicity of BA in rabbits	NTP #TER90003, 1991	125	Litter loss, malformations
CNS Developmen-tal toxicity of BA in rats; exp gd 14-17	NTP # TER90123, 1994	549	Regional or total brain weight
CNS Developmen-tal study of BA in rats; exp gd 6-15	NTP #TER93138, 1994	< 299 (0.4%)	Malformation (hydrocephalus) Embryofetal mortality

1.4 Risk assessment

Owing to the lack of any carcinogenic effect of boric acid in mice or boric acid/sodium borate in rats, no "risk assessment for carcinogenic constituents" has been performed.

1.5 Bibliography

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2 Allyl glycerol ether

2.1 Summary and overall conclusion

Allyl glycerol ether is an alkaline hydrolysis product of allyl glycidyl ether. Very limited information is available regarding the toxicity of allyl glycerol ether. A literature search identified one report addressing the genotoxicity of this and other industrial chemicals (Dean et al 1985). No other toxicity data have been identified.

In the study by Dean and coworkers, allyl glycerol ether was tested for its ability to cause gene mutations in bacteria, gene conversion in yeast and chromosomal aberrations in rat liver cells in

vitro. The highest concentrations of allyl glycerol ether investigated were essentially according to present recommendations. No evidence for any genotoxic activity was obtained.

Although there is only limited information available regarding the toxicity of allyl glycerol ether, the negative outcome in the in vitro test for genotoxic activity is reassuring. Importantly, the estimated total daily dietary concentration of allyl glycerol ether is 0.00025 ppb (Part II Section G page 4). This is 2000-fold below the level of 0.5 ppb where toxicity testing is recommended (FDA Guidance April 2002).

It is therefore concluded that the potential exposure to allyl glycerol ether from the use of the present FCS (ion exchange chromatography resin) should be of no concern with respect to human health.

2.2 Study summaries

2.2.1 Genetic toxicity studies

Gene mutations in bacteria. Allyl glycerol ether was tested in the Ames assay using the *Salmonella* strains TA 98, 100 and 1538 and the E.coli strain WP_2 and WP_2uvrA. in the absence and presence of a Aroclor 1254 induced rat liver S9 metabolizing system (Dean et al 1985). Concentrations up to 2000 µg/plate was tested. No indication of a mutagenic effect was reported.

Gene conversion in Saccharomyces. Allyl glycerol ether was also tested for the ability to induce gene conversion in *Saccharomuces Cerevisiae* (Dean et al 1985). Incubations were performed with and without a rat liver metabolizing system. The highest concentrations tested appeared to be 40 mg/mL. No indication of any gene conversion activity was reported.

Chromosomal aberrations in vitro. Allyl glycerol ether was also tested for the ability to induce chromosomal aberrations in rat liver cells *in vitro* (Dean et al 1985). Cells were grown on microscope slides contained in petri dishes. Treatment was for 24 hrs. One hundred cells from each culture was analysed microscopically. The concentrations used were up to half of the concentration causing 50% growth inhibition. No indication of any chromosomal aberrations were reported.

2.3 Bibliography

Dean BJ, Brooks TM, Hodson-Walker G and Hutson DH. (1985) Genetic toxicology testing of 41 industrial chemicals. Mutation Research 153,57-77.

3 Allyl glycidyl ether

3.1 Summary and overall conclusion

Allyl glycidyl ether has been studied for carcinogenic activity in 2-year inhalation studies in mice and rats by the National Toxicology Program (NTP TR 376, 1990). Based on the results from these studies, allyl glycidyl ether has been assigned a unit cancer risk (UCR) number of 0.0042 $(mg/kg\ b.w./day)^{-1}$ by the FDA.

The upper bound lifetime cancer risk, obtained from the UCR and the estimated daily exposure (9.08×10^{-9} mg/kg bw/day; see Part II Section G, Page 4), can therefore be estimated to 0.038×10^{-9}

A recent literature search performed on relevant databases (presented in FCN no 443) revealed no new information that would change the UCR value (set by the FDA) and hence the above estimated upper bound lifetime cancer risk.

It is concluded that the potential exposure to allyl glycidyl ether from the use of the present FCS (ion exchange chromatography resin) should be of no concern with respect to human health.

3.2 Bibliography

National Toxicology Program (NTP) Technical Report (TR) 376. (1990) Toxicology and Carcinogenesis Studies of Allyl Glycidyl Ether (CAS No. 106-92-3) in Osborne-Mendel Rats and $B6C3F_1$ Mice (Inhalation Studies). U.S. Department of Health Sevice National Institute of Health, Research Triangle Park, NC. http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/ALL_SRCH/iH_ALL_SRCH_Frames.html

4 Epichlorohydrin

4.1 Summary and overall conclusion

Epichlorohydrin is a direct acting genotoxic agent that has been studied for carcinogenic activity in rats by oral administration and by inhalation. It has been tested in mice by skin application and by subcutaneous and intraperitoneal injection (IARC 1999). Based on the observation of forestomach tumours in rats subjected to oral dosing (Konishi et al, 1980), epichlorohydrin has been assigned a unit cancer risk (UCR) number of 0.0027 $(mg/kg\ b.w./day)^{-1}$ by the FDA.

The upper bound lifetime cancer risk, obtained from the UCR and the estimated daily exposure ($9.08x10^{-9}$ mg/kg bw/day; see Part II Section G, Page 4), can therefore be estimated to 0.025×10^{-9}.

A recent literature search performed on relevant databases (presented in FCN no 443) revealed no new information that would change the UCR value (set by the FDA) and hence the above estimated upper bound lifetime cancer risk.

It is concluded that the potential exposure to epichlorohydrin from the use of the present FCS (ion exchange chromatography resin) should be of no concern with respect to human health.

4.2 Bibliography

IARC (International Agency for Research on Cancer) (1999) - Summaries & Evaluations; Epichlorohydrin. Vol 71, p603. http://www.inchem.org/documents/iarc/vol71/020-epichlorohydrin.html

Konishi Y, Kawabata A, Denda A, Ikeda T, Katada H, Maruyama H and Higashiguchi R (1989) Forestomach tumours induced by orally administered epichlorohydrin in male Wistar rats. Gann 71,922-23.

5 3-chloro-1,2-propanediol

5.1 Summary and overall conclusion

3-chloro-1,2-propanediol has been studied for carcinogenic activity by oral administration in rats (cited by Robjohns et al 2003). Based on the results from these studies, 3-chloro-1,2-propanediol has been assigned a unit cancer risk (UCR) number of 0.0087 $(mg/kg\ b.w./day)^{-1}$ by the FDA. JECFA (Joint FAO/WHO Expert Committee) has recently (2001) identified a daily tolerable intake of 2 µg/kg bw.

The upper bound lifetime cancer risk, obtained from the UCR and the estimated daily exposure ($12.7x10^{-9}$ mg/kg bw/day; see Part II Section G, Page 4), can therefore be estimated to 0.11×10^{-9}

A recent literature search performed on relevant databases (presented in FCN no 443) revealed no new information that would change the UCR value (set by the FDA) and hence the above estimated upper bound lifetime cancer risk.

It is concluded that the potential exposure to 3-chloro-1,2-propandiol from the use of the present FCS (ion exchange chromatography resin) should be of no concern with respect to human health.

5.2 Bibliography

JECFA (2001) Summary of evaluations; 3-chloro-1,2-propanediol. http://www.inchem.org/pages/jecfa.html

Robjohns S, Marshall R, Fellows M and Kowalczyk, G. (2003) In vivo genotoxicity studies with 3-monochloropropan-1,2-diol. Mutagenesis 18,401-404

6 2,3-epoxy-1-propanol (Glycidol)

6.1 Summary and overall conclusion

The toxicity of glycidol has been well characterized. The National Toxicology Program in the US have performed repeat dose toxicity studies of 13 weeks duration in mice and rats, carcinogenicity studies in the same species and a more than complete battery of genotoxicity tests. Furthermore, this program has studied the immunotoxicity of Glycidol. The embryo-fetal toxicity of glycidol in mice has been reported in the literature.

The testis and epididymis was a target organ in repeat dose toxicity studies of shorter term duration in mice and rats. The NOEL for reductions in sperm count and sperm motility were < 25 mg/kg bw/day in rats and 18 mg/kg bw/day in mice. In the same experiments but at higher doses necrosis of the cerebellum, demyelineation in the medulla of the brain occurred.

In life long studies in mice and rats, glycidol-related nonneoplastic lesions included hyperkeratosis and epithelial dysplasia of the forestomach. Fibrosis of the spleen was also present in rats.

In immunogenicity studies in female mice, glycidol was associated with immunosuppressive effects at doses of 125 mg/kg and above, a true no-effect level for glycidol in the female B6C3F1 mouse was not established since the lowest dose administered (25 mg/kg) significantly altered several parameters including erythrocyte number, hemoglobin, spleen cell number and macrophage cytotoxicity.
Embryo-fetal toxicity studies in mice revealed *no* evidence of teratogenicity at maternotoxic doses.

Glycidol was shown to cause gene mutations in bacteria, an increase in mutations in the mouse lymphoma assay, an increase in sex-linked recessive lethal mutations as well as reciprocal translocations in Drosophila, chromosomal aberrations and SCE's in CHO cells in vitro and chromosomal aberrations in vivo in mice. The positive findings obtained in vitro have occurred both with and without a metabolic activation system clearly suggesting that glycidol by itself can interfere with DNA. This is not an unexpected finding as the epoxide structure of glycidol have been shown to interact with DNA (Hemminki et al 1980; Segal et al 1990). It can thus be concluded that there is clear evidence for the mutagenic/genotoxic activity of glycidol.
Life-long studies in male F344/N rats showed *clear evidence* of carcinogenic activity based on increased incidences of mesotheliomas of the tunica vaginalis; fibroadenomas of the mammary gland; gliomas of the brain; and neoplasms of the forestomach, intestine, skin, Zymbal gland, and thyroid gland. There was *clear evidence* of

***carcinogenic activity* for female F344/N rats, based on increased incidences of fibroadenomas and adenocarcinomas of the mammary gland; gliomas of the brain; neoplasms of the oral mucosa, forestomach, clitoral gland, and thyroid gland; and leukemia.**

Similar studies in $B6C3F_1$ mice revealed clear evidence *of carcinogenic activity* for male animals based on increased incidences of neoplasms of the harderian gland, forestomach, skin, liver, and lung. There was *clear evidence of carcinogenic activity* for female $B6C3F_1$ mice, based on increased incidences of neoplasms of the harderian gland, mammary gland, uterus, subcutaneous tissue, and skin. Other neoplasms that may have been related to the administration of glycidol were fibrosarcomas of the glandular stomach in female rats and carcinomas of the urinary bladder and sarcomas of the epididymis in male mice.

Estimation of Unit Cancer Risk (UCR) according to FDA guidance revealed that glycidol had the highest carcinogenic potency in male rats; 0.025 $(mg/kg\ b.w./day)^{-1}$ by the FDA (see section 6.4 Risk Assessment).

The upper bound lifetime cancer risk, obtained from the UCR and the estimated daily exposure to glycidol ($90.8x10^{-9}$ mg/kg bw/day; see Part II Section G, Page 4), can therefore be estimated to 2.3×10^{-9}.

It is therefore concluded that the potential exposure to glycidol from the use of the present FCS (ion exchange chromatography resin) should be of no concern with respect to human health.

6.2 Study summaries

6.2.1 Genetic toxicity studies

The studies summarized below are all obtained from the NTP study report on glycidol, NTP TR 374.

Gene mutations in bacteria: Glycidol was tested in the Ames Salmonella assay using the preincubation protocol with tester strains TA97, TA98, TA 100, TA 1535 and TA1537 with and without a metabolic activation system (Canter et al. 1986; NTP TR 374). Concentrations of up to 10,000 µg/plate was used. Glycidol was found to be mutagenic in all tester strains both with and without a metabolic activation system.

Gene mutations and chromosomal translocation in Drosophila Melanogaster: Glycidol was investigated for its ability to cause sex-linked recessive lethal mutations in Drosophila by feeding adult Canton-S wild type young males (no more than 24 hrs old). Glycidol was also assayed for induction of reciprocal translocations. 1230 ppm of glycidol in the feed caused an 8-fold increase in lethal mutations. The same concentration (in the feed) resulted in an 1.8-fold increase in translocations.

Genetic toxicity in mouse L5178Y/TK lymphoma cells: Glycidol was tested in the mouse lymphoma assay with and without a metabolic activation system (NTP TR 374). Incubations with glycidol were performed for 4 hours whereafter the cells without the chemical were incubated for another 48 hours to allow expression of the mutant phenotype. Concentrations up to 10 nL/mL was found to give an acceptable survival. An increase in Tft (trifluoro-thymidine) -resistent cells was observed at concentrations from 1.25 nL/mL (approx. 1.25 µg/mL) and upwards.

Chromosomal aberrations and sister chromatid exchanges in CHO cells: Glycidol was tested for induction of chromosomal aberrations and sister chromatid exchanges in Chinese Hamster Ovary (CHO) cells both with and without a metabolic activation system (NTP TR 374). The highest concentration investigated was 400 µg/mL in the test for chromosomal aberrations and 150 µg/mL in the test for SCE's.

Concentrations of 12.5-100 µg/mL caused a clear induction of chromosomal aberrations in the absence of a rat S9 mix whereas 200-400 µg/mL caused a clear induction of aberrations in the presence of a rat S9 mix. Since pronounced inductions of genotoxic activity was observed at the lowest concentrations tested, it is likely that also lower concentrations would produce an increase in chromosomal aberrations.

In the absence of a rat S9 mix, concentrations of 1.1-15 µg/mL caused a clear induction (6-fold at the lowest concentration) of SCE's per cell whereas in the presence of an S9 mix, concentrations between 11.1-150 µg/mL caused a clear induction of SCE's.

Chromosomal aberrations in vivo: The ability of glycidol to induce chromosomal aberrations in vitro was investigated in male mice (NTP TR 374). Groups of mice were given by i.p. injection either the vehicle, 37.5, 75, 150 or the positive control compound (mitomycin C) twice with a 24 hr interval between the doses. Twenty-four hours after the second injection blood smears were prepared and 2000 polychromatic erythrocytes were scored for the presence of micronucleated cells. Two trials were run. The incidence of PCE's in high dose animals (150 mg/kg) was approximately 3 times the incidence in vehicle control animals. Mitomycin C showed the expected result.

Overall conclusion on the genotoxicity of glycidol

A large number of studies is available on the genotoxicity of glycidol and many of these studies were conducted prior to the National Toxicology Program studies on glycidol was initiated. The results of these earlier studies are summarized in NTP TR 374. Since the NTP studies clearly show that glycidol possess genotoxic activity an overview of the studies carried out prior to the NTP studies has not been included in this review.

Glycidol has been shown to cause gene mutations in bacteria, an increase in mutations in the mouse lymphoma assay, an increase in sex-linked recessive lethal mutations as well as reciprocal translocations in Drosophila, chromosomal aberrations and SCE's in CHO cells in vitro and chromosomal aberrations in vivo in mice. The positive findings obtained in vitro have occurred both with and without a metabolic activation system clearly suggesting that glycidol by itself can interfere with DNA. This is not an unexpected finding as the epoxide structure of glycidol have been shown to interact with DNA (Hemminki et al 1980; Segal et al 1990). It can thus be concluded that there is clear evidence for the mutagenic/genotoxic activity of glycidol.

6.2.2 In vivo toxicity tests

Acute and repeat dose toxicity studies

*Acute toxicity studies***: The oral LD50 in rats has been reported to 850 mg/kg (Sax 1979) whereas that after inhalation (8 hrs) in rats and dermal exposure in rabbits has been determined to 580 ppm and 1980 mg/kg, respectively.**

Sixteen-day toxicity study in mice and rats: Glycidol was given by gavage to groups of five rats or five mice of each sex at daily doses of 37.5, 75, 150, 300 or 600 mg/kg; vehicle controls received distilled water (NTP TR 374). All rats that received 600 mg/kg died between days 3 and 13. Edema and degeneration of the epididymal stroma, atrophy of the testis, and granulomatous inflammation of the epididymis occurred in males that received 300 mg/kg.

All mice that received 600 mg/kg and two males and two females that received 300 mg/kg died by day 4 of the studies. Focal demyelination in the medulla and thalamus of the brain occurred in all female mice that received 300 mg/kg.

Thirteen-week studies in mice and rats: The doses to rats (10/group/sex) were 25, 50, 100, 200 or 400 mg/kg, and doses to mice (10/group/sex) were 19, 38, 75, 150 or 300 mg/kg; vehicle

controls received distilled water (NTP TR 374). All rats that received 400 mg/kg died by week 2; three males and one female that received 200 mg/kg died during weeks 11-12. Final mean body weights of male rats that received 50, 100, or 200 mg/kg were 96%-85% that of vehicle controls; final mean body weights of female rats receiving the same doses were 95%-89% that of vehicle controls. Sperm count and sperm motility were reduced in male rats that received 100 or 200 mg/kg. Necrosis of the cerebellum, demyelineation in the medulla of the brain, tubular degeneration and/or necrosis of the kidney, lymphoid necrosis of the thymus, and testicular atrophy and/or degeneration occurred in rats that received 400 mg/kg.

All mice that received 300 mg/kg died by week 2; deaths of mice that received 150 mg/kg occurred during weeks 4-8 for males and weeks 1-5 for females. Mean body weights of chemically exposed mice surviving to the end of the studies were generally 90%-94% those of vehicle controls. Sperm count and sperm motility were reduced in dosed male mice. Compound-related histopathologic lesions included demyelination of the brain in males and females that received 150 or 300 mg/kg, testicular atrophy in males at all doses, and renal tubular cell degeneration in male mice that received 300 mg/kg. Based on reduced survival, reduced weight gain, and histopathologic lesions in the brain and kidney in rats that received 200 or 400 mg/kg and on reduced survival and histopathologic lesions of the brain in mice that received 150 or 300 mg/kg, doses selected for the 2-year carcinogenicity studies of glycidol were 37.5 and 75 mg/kg for rats and 25 and 50 mg/kg for mice.

Reproduction toxicity studies

Embryo-fetal toxicity in mice. Pregnant outbred albino mice (CD-1) were given glycidol by gastric intubation on d 6-15 of gestation (Marks et al 1982). The mice were killed on d 18 and the offspring checked for gross, visceral, and skeletal malformations. Glycidol showed no evidence of teratogenicity. There was a significant increase in the number of stunted fetuses at 200 mg/kg bw/day but all of these were present in a single litter. This dose was overtly maternotoxic as it was lethal in 5 of 30 dams.

Carcinogenicity studies

Carcinogenicity studies in rats. Groups of 50 F344/N rats/sex were administered by gavage glycidol at 0 (vehicle control), 37.5 or 75 mg/kg body weight for 2 years (NTP TR 374).

The mean body weights of glycidol exposed male rats generally ranged from 80% to 94% of those of vehicle controls, and mean body weights of glycidol exposed female rats were from 90% to 97% those of vehicle controls. Virtually all male and female rats that received glycidol died or were killed in a moribund condition as a result of the early induction of neoplastic disease (final survival--male: vehicle control, 16/50; low dose, 0/50; high dose, 0/50; female: 28/50; 4/50; 0/50). Survival of vehicle control male rats was lower than that usually observed but specific causes of deaths could not be determined.

Glycidol-related nonneoplastic lesions included hyperkeratosis and epithelial dysplasia of the forestomach. Fibrosis of the spleen was also present in rats of each sex. Exposure to glycidol induced dose-related increases in the incidences of neoplasms in numerous tissues (see summary Tables under the Risk Assessment heading below or summary Table on page 5 of the Technical Report). In male rats, mesotheliomas arising in the tunica vaginalis and frequently metastasizing to the peritoneum were considered the major cause of early death. Early deaths in female rats were associated with the presence of mammary gland neoplasms.

Under the conditions of this 2-year gavage study, there was clear evidence of carcinogenic activity of glycidol for male F344/N rats, based on increased incidences of mesotheliomas of the tunica vaginalis; fibroadenomas of the mammary gland; gliomas of the brain; and neoplasms of the forestomach, intestine, skin, Zymbal gland, and thyroid gland. There was clear evidence of

carcinogenic activity for female F344/N rats, based on increased incidences of fibroadenomas and adenocarcinomas of the mammary gland; gliomas of the brain; neoplasms of the oral mucosa, forestomach, clitoral gland, and thyroid gland; and leukemia.

Carcinogenicity study in mice. Groups of 50 B6C3F1 mice/sex were administered by gavage glycidol at doses of 0 (vehicle control), 25 or 50 mg/kg body weight for 2 years (NTP TR 374).

Mean body weights of chemically exposed male mice were similar to those of vehicle controls; mean body weights of chemically exposed female mice were 79%-95% of those of vehicle controls. The survival of male mice and low dose female mice was similar to that of vehicle controls; survival of female mice that received 50 mg/kg was lower than that of vehicle controls after week 101 (final survival--male: 33/50; 25/50; 27/50; female: 29/50; 27/50; 17/50).

Chemical-related nonneoplastic lesions in mice included hyperkeratosis and epithelial dysplasia of the forestomach. Cysts of the preputial gland and kidney were present in male mice. Exposure to glycidol induced dose-related increases in the incidences of neoplasms in numerous tissues in mice (see summary Tables under the heading of Risk Assessment below or summary Table on page 5 of the Technical Report).

This study showed clear evidence of carcinogenic activity for male B6C3F1 mice based on increased incidences of neoplasms of the harderian gland, forestomach, skin, liver, and lung. There was clear evidence of carcinogenic activity for female B6C3F1 mice, based on increased incidences of neoplasms of the harderian gland, mammary gland, uterus, subcutaneous tissue, and skin. Other neoplasms that may have been related to the administration of glycidol were fibrosarcomas of the glandular stomach in female rats and carcinomas of the urinary bladder and sarcomas of the epididymis in male mice.

Other studies

Immunogenicity studies in female mice. The effect of glycidol on the immune system was investigated in female B6C3F1 mice (NTP Study No. IMM91019). The animals were administered glycidol by gavage daily for 14 days at doses of 25, 125 and 250 mg/kg. Mice exposed to glycidol at doses up to and including 250 mg/kg did not have significant decreases in body weight or body weight gain when evaluated over the two-week exposure period. While the brain, thymus, spleen and lungs were unaffected by the glycidol exposure, an increasing trend was observed in liver weights. Additionally, kidney weights were increased (42%) in the glycidol exposed animals dose dependently. No statistically significant effects were observed on leukocyte numbers, leukocyte differentials, reticulocytes, mean corpuscular volume, mean corpuscular hemoglobin or mean corpuscular hemoglobin concentrations. A slight, albeit statistically significant, decrease was observed in the erythroid elements, erythrocytes (4%), hemoglobin (4%), and hematocrit (5%) which was dose related.

Table 6.1 summarizes the immunology studies. Exposure to glycidol decreased the number of B cells (23%) and decreased the number of CD4+CD8- (15%) in the T cell subsets. Total T cells and the other T cell subsets were not affected. Glycidol produced a dose-dependent decrease (41%) in the antibody-forming cell response to sheep erythrocytes. The proliferative response to mitogens, both Con A and LPS, was not affected. However, a decreasing trend in the proliferative response to F(ab)2+BSF-1 was observed. The proliferative response to allogeneic cells as evaluated in the MLR was not affected and overall the CTL response was not affected. A dose-dependent decrease was observed in the natural killer cell activity (29%) when evaluated at the highest (25:1) effector:target ratio. An increase in cytotoxicity of both resident macrophages alone and resident macrophages stimulated with gamma interferon was observed in animals receiving low dose glycidol exposure. No effect was observed on macrophage cytotoxicity at the middle and high dose groups. The peritoneal cell numbers were not affected at any dose level.

Table 6.1. Summary Table for Immunology Studies

Parameter	Results	Max Effect	Dose	Comment
Surface Markers				
Ig^+	Decreased	23%	250 mg/kg	Dose Response
Thy 1.2^+	No Effect			
$CD4^+CD8^-$	Decrease	15%	250 mg/kg	Dose Response
$CD4^-CD8^+$	No Effect			
$CD4^+CD8^+$	No Effect			
Spleen IgM Antibody-Forming Cell Response to Sheep Erythrocytes				
IgM AFC to sRBC Cells	Decreased	41%	250 mg/kg	Dose Response
Proliferation Assays, i.e. Mixed Leukocyte Response				
Con A	No Effect			
LPS	No Effect			
$F(ab^1)_2$+BSF-1	Decreased	16%	125 mg/kg	Middle Dose Only
Medium	Increased	45%	25 mg/kg	Mitogen Assay Only
MLR	No Effect			
Cytotoxic T Lymphocyte Activity				
CTL	No Effect			
NK Cell Activity				
1:100	Decreased	31%	125 mg/kg	Dose Response
1:50	Decreased	36%	250 mg/kg	Dose Response
1:25	Increased	79%	25 mg/kg	Low Dose Only
Macrophage Activity				
Resident				
Macrophage %	Increased	151%	25 mg/kg	Low Dose Only
+Gamma Interferon	Increased	68%	25 mg/kg	Low Dose Only
PE Cell Number	No Effect			

In the three host resistance studies conducted, host resistance to Listeria monocytogenes was not affected, while an increase in host resistance to Streptococcus pneumoniae and a decrease in host resistance to the B16F10 Melanoma tumor model was observed (Table 6.2).

Table 6.2. Summary Table for Host Resistance Studies.

Parameter	Results	Maximum Effect	Dose	Comment
Listeria monocytogenes	No Effect			
Streptococcus pneumoniae	Increased Resistance	50%	250 mg/kg	High Challenge
B16F10 Melanoma	Decreased Resistance	129% Increase in Tumor Burden	250 mg/kg	Dose Response

In summary, while most of the immunosuppressive effects resulting from glycidol exposure were observed at the 125 mg/kg and above dose levels, a true no-effect level for glycidol in the female B6C3F1 mouse could not be established since the lowest dose administered significantly altered several parameters including erythrocyte number, hemoglobin, spleen cell number and macrophage cytotoxicity.

6.3 NOEL's

Table 6.3 below depicts the NOEL's relating to non-neoplastic findings from the repeat dose toxicity, immunogenicity study, and carcinogenicity studies conducted by the NTP (NTP TR 374; NTP Study No IMM91019).

Table 6.3. NOEL's of non-neoplastic findings of Glycidol.

Study type and species	NOEL (mg/kg bw/day)	Effect
13-week Rat	< 25	Sperm count
13-week Mice	19	Sperm count
Carc study Rat	< 38	hyperkeratosis and epithelial dysplasia of the forestomach
Carc study Mice	< 25	hyperkeratosis and epithelial dysplasia of the forestomach
Immunog. Mice	< 25	Macrophage cytotox

The NOEL's with regard the major toxicities identified in the repeat dose toxicity, carcinogenicity and immunogenicity studies with glycidol are given in Table 6.4 below.

Table 6.4. NOEL's of major effects identified in repeat dose studies with glycidol.

Study type and species	NOEL (mg/kg bw/day)	Effect
13-week Rat	< 25	Sperm count
	200 M; 100 F	Cerebellar necrosis
	100	Testicular atrophy
	200 M/F	Renal tubular deg./necrosis
	200 F	Thymic Lymphoid necrosis
13-week Mice	19	Sperm count
	150	Renal tubular deg.
	75 M; 150 F	Brain demyelination
Carcinogen Rat	< 38	hyperkeratosis and epithelial dysplasia of the forestomach
	< 38	fibrosis of the spleen
Carcinogen Mice	< 25	hyperkeratosis and epithelial dysplasia of the forestomach
Immunotox Mice	< 25	Macrophage cytotoxicity
	125	Decrease Ab-forming cell response to sheep erythrocytes

It is concluded that in repeat dose studies in mice and rats the most sensitive target organ is the testis with NOEL's with regard to sperm count reductions at daily doses of 19 and less than 25 mg/kg bw/day, respectively. Forestomach toxicity has been observed at the lowest dose investigated in life long studies in mice and rats (25 and 38 mg/kg bw/day). In the same rat study, toxicity to the spleen (fibrosis) was also observed at the lowest dose investigated. Functional effects on the immune system may occur at lower doses than observed in the mice study (25 mg/kg) since the duration of this study was only 2 weeks.

In view of the clear carcinogenic potential of glycidol, non-neoplastic lesion are not considered critical in the risk assessment of human exposure to low doses of glycidol.

6.4 Risk assessment

The tumour incidence in **male rats** from the NTP study (NTP TR 374) is given in Table 6.5 below.

Table 6.5. Tumor incidence in male rats.

Tumour type	Glycidol (mg/kg bw/day)					
	0		38		75	
	Incidence	%	Incidence	%	Incidence	%
Mesothelium; abdom cav/tunica vaginalis	3/50	6	34/50*	68	39/50*	78
Mammary gland; fibroadenoma	3/50	6	8/50	16	7/50*	14
Brain glioma	0/50	0	5/50*	10	6/50*	12
Forestomach; sq cell papilloma/carcinoma	1/50	2	2/50	4	6/50*	12
Intestines; adenomatous polyps and adenc.	0/50	0	1/50	2	4/50	8
Skin; seb gland aden/ade car, basal cell carc	0/50	0	5/50*	10	4/50*	8
Zymbal gland carcinoma	1/50	2	3/50	6	6/50	12
Thyroid gland follicular cell aden/carcinom	1/50	2	4/50	8	6/50*	12

* Statistical significance $p < 0.05$ (Fisher Exact Test)

The Unit Cancer Risk derived from **male rat** tumours = 31/50 / 38 mg/kg bw/day + 4/50 / 75 mg/kg bw/day + 5/50 / 38 mg/kg bw/day + 5/50 / 75 mg/kg bw/day + 5/50 / 38 mg/kg bw/day + 5/50 / 75 mg/kg bw/day = 0.025299 mg/kg bw/day^{-1}.

The tumour incidence in **female rats** from the NTP study (NTP TR 374) is given in Table 6.6 below.

Table 6.6. Tumor incidence in female rats.

Tumour type	Glycidol (mg/kg bw/day)					
	0		38		75	
	Incidence	%	Incidence	%	Incidence	%
Clitoral gland adenoma, adenocar, carcinoma	5/50	10	9/50	18	12/50*	24
Mammary gland; fibroadenoma/adenocarc	14/50	28	34/50*	68	37/50*	74
Brain glioma	0/50	0	4/50	8	4/50	8
Forestomach; sq cell papilloma/carcinoma	0/50	0	4/50*	8	11/50*	22
Mouth/tongue sq cell papilloma/carcinoma	1/50	2	3/50	6	7/50	14
Skin; seb gland aden/ade car, basal cell carc	0/50	0	5/50*	10	4/50*	8
Hematopoietic mononucl cell leukemia	13/50	26	12/50	28	20/50*	40
Thyroid gland follicular cell aden/carcinom	0/50	0	1/50	2	3/50	6

* Statistical significance $p < 0.05$ (Fisher Exact Test)

The Unit Cancer Risk derived from **female rat** tumours = 7/50 / 75 mg/kg bw/day + 20/50 / 38 mg/kg bw/day + 4/50 / 38 mg/kg bw/day + 5/50 / 38 mg/kg bw/day + 7/50 / 75 mg/kg bw/day = 0.018996 mg/kg bw/day^{-1}.

The tumour incidence in **male mice** from the NTP study (NTP TR 374) is given in Table 6.7 below.

Table 6.7. Tumor incidence in male mice.

Tumour type	Glycidol (mg/kg bw/day)					
	0		25		50	
	Incidence	%	Incidence	%	Incidence	%
Harderian gland adenoma adenocarcinoma	8/46	17	12/41	29	22/44*	50
Hepatocellular adenoma/carcinoma	24/50	48	31/50	62	35/50*	70
Skin; squamous cell papilloma	0/50	0	0/50	0	4/50*	8
Forestomach; sq cell papilloma/carcinoma	1/50	2	2/50	4	10/50*	20
Lung alveolar/bronchiolar adenoma/carcin	13/50	26	11/50	22	21/50*	42

* Statistical significance $p < 0.05$ (Fisher Exact Test or Incidental Tumour Test)

The Unit Cancer Risk derived from **male mice** tumours = 0.37 / 50 mg/kg bw/day + 0.22 / 50 mg/kg bw/day + 0.08 / 50 mg/kg bw/day + 0.18 / 50 mg/kg bw/day + 0.16 / 50 mg/kg bw/day = 0.0202 mg/kg bw/day^{-1}.

The tumour incidence in **female mice** from the NTP study (NTP TR 374) is given in Table 6.8 below.

Table 6.8. Tumor incidence in female mice

Tumour type	Glycidol (mg/kg bw/day)					
	0		25		50	
	Incidence	%	Incidence	%	Incidence	%
Harderian gland adenoma/adenocarcinoma	4/46	9	11/43*	26	17/43*	40
Mammary gland; fibroadenoma/adenocarc	2/50	4	6/50	12	15/50*	30
Subcutaneous sarcoma/fibrosarcoma	0/50	0	3/50	6	9/50*	18
Hepatocellular adenoma/carcinoma	9/50	18	7/50	14	14/50	22
Hemangioamas/hemangiosarcoma	1/50	2	3/50	6	5/50	10

* Statistical significance $p < 0.05$ (Fisher Exact Test or Incidental Tumour Test)

The Unit Cancer Risk derived from **female mice** tumours = 0.17 / 25 mg/kg bw/day + 0.26 / 50 mg/kg bw/day + 0.18 / 50 mg/kg bw/day = 0.0156 mg/kg bw/day^{-1}.

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7 Bromine

7.1 Summary and overall conclusion

Bromine can leach from the FCS resulting in an estimated maximal daily intake of 0.0727 ng/kg bw/day. In US, bromine is registered for use in water filters to purify drinking water aboard U.S. Naval ships and offshore oil well platforms. It also is used as a general disinfectant and sanitizer in indoor, non-food contact areas such as commercial establishments, hospitals and households, to control bacteria and fungi.

The US EPA established a food additive tolerance for 1.0 ppm residual bromine in potable water aboard Naval surface ships in 1976 and confirmed in 1993 that this level provides an adequate margin of safety to protect the public health. A daily intake of 2 L of water at the 1.0 ppm threshold level would result in a daily exposure to bromine of 2 mg (0.03 mg/kg bw/day for a person weighing 70 kg). This level of acceptable bromine exposure is thus more than 5 orders of magnitude greater than the estimated daily intake of bromine originating from the FCS.

Bromine may react with other compounds in water and living organisms to form bromides and also to a small extent bromates. However, as the potential additional exposure to bromide and bromate caused by such conversion is small compared to the bromide and bromate exposures dealt with elsewhere in the filing (see Section 8 below and Part III, Safety Narrative), they are not further considered here.

7.2 Study summaries

The reactivity of bromine in biological systems makes it difficult to separate the effects of the bromine from those of the bromine compounds and metabolites. Inhalation of the irritant bromine vapours and/or direct contact (liquid or vapour) with skin and mucous membranes will produce direct tissue injury. Injury may occur at various levels of the respiratory tract depending on the concentration of bromine and duration of exposure. Target organs include the upper and lower respiratory tract, skin, and eyes. In theory, although never demonstrated in the literature, the potential exists for bromine to accumulate in body tissues as bromide after an inhalation or direct skin contact. If accumulated to sufficiently high concentrations, bromide would subsequently produce neurotoxic and acne-like effects as seen with the ingestion of bromide compounds or the chronic inhalation of methyl bromide. Owing to these characeteristics it is not surprising that little toxicity information is available on orally administered bromine.

7.2.1 Genetic toxicity studies

No data have been found implicating bromine as a mutagen (Calabrese & Kenyon, 1991).

7.2.2 In vivo toxicity studies

Rats fed 0.01 mg/kg of bromine for 6 months experienced changes in their conditioned reflexes and several blood indices (HSDB Bromine).

No data have been found implicating bromine as a teratogen (Calabrese & Kenyon, 1991). No data have been found implicating bromine as a carcinogen (Calabrese & Kenyon, 1991; Alderson, 1986).

7.3 NOEL's

There are no repeat dose toxicity studies and no genotoxicity or carcinogenicity studies conducted with oral administration of bromine and thus no NOEL's can be derived. However, an Acceptable Daily Intake (ADI) value after oral ingestion of 1 mg/kg body weight have been referenced (Sticht & Kaferstein, 1988), a value identical to the 1.0 ppm food additive tolerance level established by the US EPA for residual bromine in potable water aboard Naval surface ships (40 CFR § 185.425) (EPA R.E.D Facts Bromine 1993). In 1993 the EPA confirmed that this level provides an adequate margin of safety to protect the public health.

7.4 Risk Assessment

Based on the established food additive tolerance of 1.0 ppm residual bromine in potable water one can calculate that a daily intake of 2 L of such water would result in a daily exposure to bromine of 2 mg (0.03 mg/kg bw/day for a person weighing 70 kg). This level of acceptable bromine exposure is thus more than 5 orders of magnitude greater than the estimated daily intake of bromine (0.0727 ng/kg bw) originating from the FCS.

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8 Sodium bromate

8.1 Summary and overall conclusion

The estimated maximal daily intake of sodium bromate from the use of the FCS is 0.116 ng/kg bw. Sodium bromate is not regulated as such but potassium bromate is approved for use as a food additive in the malting of barley (21 CFR 172.730). The limitation given is that the final level in the beverage produced should be less than 25 ppm.

The US EPA have made a "chronic health hazard assessment for noncarcinogenic effects" and a "carcinogenicity assessment for lifetime exposure" of the bromate ion (BrO_3^-). In assessing the noncarcinogenic effect they established an oral Reference Dose (RfD) which is an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD was determined to

0.004 mg/kg bw/day of BrO_3^- and was based on observations (LOAEL) of urothelial hyperplasia at a dose of 6.1 mg BrO_3^- kg bw/day and the absence of such an effect (NOAEL) at the dose of 1.1 mg BrO_3^- kg bw/day in a life-time carcinogenicity study in rats where potassium bromate was administered in the drinking water. An uncertainty factor of 300 was used to estimate the RfD.

In assessing the carcinogenicity of a life time exposure to the bromate ion, the Agency reviewed three life-long studies in rats with exposure of potassium bromate via the drinking water. All studies were judged as being well conducted and with an adequate number of animals. Tumors were observed at multiple sites, including the kidney (adenomas and carcinomas), the thyroid (follicular cell adenomas and carcinomas), and the peritoneum (mesotheliomas). Whereas male rats had tumors at all three sites, only kidney tumors were observed in female rats. The kidney tumors in female rats developed in the absence of the significant toxicity observed in the male rats.

The weight-of-evidence judgment was thus that the bromate ion is a likely human carcinogen by the oral route of exposure. A quantitative estimate of the carcinogenic risk from oral exposure gave a Slope Factor of 0.7 i.e. risk per (mg/kg bw)/day whereas the drinking water Unit Risk (UR) was 2×10^{-5} per µg/L. An excess cancer risk of 1 in a million was estimated to occur at a drinking water concentration of 0.05 µg/L. Assuming a daily intake of 2 L of water, this would translate into a daily oral dose of 100 ng and on a ng/kg bw basis, based on a body weight of 70 kg, an exposure of approximately 1.4 ng/kg bw. The RfD value of 4000 ng/kg bw/day as well as this latter "acceptable daily exposure" from a carcinogenic risk point of view (1.4 ng/kg bw) are both above the estimated maximal daily intake of bromate ion (0.099 ng/kg bw/day or 0.116 ng/kg bw/day of sodium bromate) that originates from the FCS. Moreover, these latter estimates are well below the levels (25 ppm) of potassium bromate or bromate ion accepted in beverages. There should thus be no concern from a health perspective with any such exposure to the bromate ion.

8.2 Study Summaries

8.2.1 Genetic toxicity studies (US EPA IRIS on bromate)

The genotoxicity of bromate has been evaluated in a variety of in vitro and in vivo systems. It has tested positive in the *Salmonella typhimurium* assay in the presence of metabolic activation and in an in vitro test for chromosomal aberrations that uses Chinese hamster fibroblasts (Ishidate et al., 1984). Dose-dependent increases in the number of aberrant metaphase cells were observed following single oral doses of potassium bromate to Long-Evans rats (Fuji et al., 1988). Bromate caused significant increases in the number of micronuclei following either i.p. injection (Hayashi et al., 1988; Awogi et al., 1992) or gavage dose (Hayashi et al., 1989; Nakajima et al., 1989) in mice. Also, i.p. injection of bromate in F344 rats resulted in significantly increased micronuclei in reticulocytes (Sai et al., 1992). Bromate was cytotoxic, increased the frequency of cells with micronuclei, increased the number of chromosome aberrations, and increased DNA migration in a series of assays that used V79 Chinese hamster cells (Speit et al., 1999). Furthermore, potassium bromate clearly induced gene mutations at the HPRT locus of V79 Chinese hamster cells (Speit et al., 1999).

8.2.2 In vivo toxicity studies

Principal study for assessment of non-carcinogenic risk (US EPA IRIS on bromate)

The US EPA considered that the prinicpal in vivo study for setting the RfD for bromate ion was a carcinogenicity study in male rats (DeAngelo et al. (1998). F344 rats were administered potassium bromate in drinking water at concentrations of 0, 0.02, 0.1, 0.2, and 0.4 g/L (78/group), for 100 weeks. Time-weighted mean daily doses were calculated by the authors from

the mean daily water consumption and the measured concentrations of potassium bromate. Bromate doses for the rats were 0, 1.1, 6.1, 12.9, and 28.7 mg BrO_3^-/kg-day. For rats, 6 animals/group were included for interim sacrifices, which occurred at 12, 26, 52, and 77 weeks. Parameters evaluated included survival, body weight, organ weight, serum chemistry, and histopathology.

An observed decrease in survival and body weight was attributed to an excessive mesothelioma burden. The effects of potassium bromate on survival and body weight in rats indicate that the maximum tolerated dose (MTD) was reached in this study.

The study showed a significant dose-dependent increase in the incidence of urothelial hyperplasia in rats in dose groups of 6.1 mg/kg-day and higher. The authors also observed foci of mineralization of the renal papilla and eosinophilic droplets in the proximal tubule epithelium, although no information on the dose levels for these findings was presented.

Principal studies for assessment of carcinogenic risk (US EPA IRIS on bromate)

Under the current *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986), bromate would be classified as B2, probable human carcinogen. Under the *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996), bromate should be evaluated as a likely human carcinogen by the oral route of exposure.

Although no epidemiological studies or studies of long-term human exposure to bromate are available, bromate is carcinogenic to male and female rats following exposure in drinking water. Three key studies (Kurokawa et al., 1986a, 1986b; DeAngelo et al., 1998) demonstrate the carcinogenicity of bromate in rats. All studies were well conducted, with an appropriate route of exposure and adequate numbers of animals. Several aspects of these bioassay studies support the conclusion that bromate has the potential to be a human carcinogen. First, tumors were observed at multiple sites, including the kidney (adenomas and carcinomas), the thyroid (follicular cell adenomas and carcinomas), and the peritoneum (mesotheliomas). In DeAngelo et al. (1998) the mesotheliomas arose from the *tunica vaginalis testis* and spread throughout the peritoneal cavity on the serosal surfaces of many organs. Kurokawa et al. (1986a, 1986b) do not specify the origin of the peritoneal mesotheliomas observed. Whereas male rats had tumors at all three sites, only kidney tumors were observed in female rats. However, the kidney tumors in female rats developed in the absence of the significant toxicity observed in the male rats.

Second, a clear dose-response relationship existed in tumor incidence and in severity/progression of tumors. Kurokawa (1986a) observed a progression in severity from renal dysplastic foci, a preneoplastic lesion, through renal adenomas to renal carcinoma as the dose increased. Kurokawa et al. (1986b) observed dose-response relationships for kidney tumors in both male and female rats. Kurokawa (1986a) observed dose-response relationships for two other tumor types, mesotheliomas and thyroid follicular cell, in male rats. DeAngelo et al. (1998) observed dose-response relationships for all three tumor types in rats.

8.3 NOEL's

On the basis of kidney effects observed in male rats in a carcinogenicity study with postassium bromate (DeAngelo et al., 1998), a NOAEL of 1.1 mg BrO_3^-/kg-day and a LOAEL of 6.1 mg BrO_3^-/kg-day can be derived.

No NOEL was observed in the carcinogenicity study by De Angelo et al. (1998). Table 8.1 below depicts the dose and the observed tumour incidence.

Table 8.1. Carcinogenicity results.

Incidence[a]				
Administered dose (mg BrO_3^-/kg-day)	**Human Equivalent Dose[b] (mg BrO_3^-/kg-day)**	**Testicular Mesothelioma**	**Thyroid Follicular Adenoma and Carcinoma**	**Kidney Adenoma and Carcinoma**
0	0	0/71	0/60	1/69
1.1	0.3	4/73	4/63	1/67
6.1	1.7	5/73	2/67	6/71
12.9	3.5	11/71	5/58	3/62
28.7	7.9	31/67	17/54	18/56

[a]Includes tumor incidences from interim sacrifice groups.
[b]Human equivalent dose was estimated using body weight to the 0.75 power.

8.4 Risk Assessment

For the assessment of non-carcinogenic risk, a reference dose (RfD) can be estimated based on the NOAEL multiplied by an uncertainty factor. EPA used an uncertainty factor of 10 to account for extrapolating from animals to humans and another factor of 10 was used to protect sensitive subpopulations and to account for potential differences between adults and children. A factor of 3 was used to account for some deficiencies in the database. The bromate database consists of chronic and subchronic studies in rats and mice and a screening-level reproductive/ developmental study in rats. The database is missing developmental studies in two species and a multigeneration study. This resulted in a total uncertainty factor of 300 and a RfD of 0.004 mg/kg/day of BrO_3^-. The RfD value for bromate ion is well above the estimated maximal daily intake of bromate ion (0.099 ng/kg/day) that originates from the FCS.

A quantitative estimate of the carcinogenic risk (oral exposure) based on the data from DeAngelo Slope Factor of 0.7 i.e. risk per (mg/kg)/day whereas the drinking water Unit Risk (UR) was 2×10^{-5} per µg/L. An excess cancer risk of 1 in a million was estimated to occur at a drinking water concentration of 0.05 µg/L. A daily intake of 2 L of water, would translate into a daily oral dose of bromate ion of 100 ng and on a ng/kg basis, based on a body weight of 70 kg, an exposure of approximately 1.4 ng/kg. This “acceptable daily exposure” from a carcinogenic risk point of view is above the estimated maximal daily intake of bromate ion (0.099 ng/kg/day) that originates from the FCS.

The estimated maximal daily intake of bromate ion (0.099 ng/kg/day) that originates from the FCS is also way above levels (25 ppm) of potassium bromate or bromate ion accepted in beverages. There should thus be no concern from a health perspective with any exposure to the bromate ion originating from the FCS.

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9 Bromoacetic acid

9.1 Summary and overall conclusion

Bromoacetic acid can be formed in waters that contain bromide and it may also be a by-product of drinking water disinfection where coastal waters contain significant levels of bromine. The estimated maximal daily intake of bromoacetic acid from the use of the FCS is 0.127 ng/kg.

There are limited data available on the toxicity of bromoacetic acid. Acute oral toxicity studies in rats have shown LD50 values below and above 50 mg/kg. Studies on repeat dosing in animals are essentially absent. Some reproductive toxicity data is available with evidence of developmental toxicity at 100 mg/kg bw in rats and with a NOAEL at 50 mg/kg bw. Based on this NOAEL and the use of an uncertainty factor of 1000, an oral reference dose (RfD; an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious non-carcinogenic effects during a lifetime) of 50 µg/kg bw is obtained. Such a RfD is thus more than 5 orders of magnitude higher than the estimated maximal daily exposure to bromoacetic from the use of the FCS.

Of perhaps more relevance when assessing the potential risk associated with exposure to bromoacetic acid is it documented genotoxic activity. Several reports are avilable on the positive effects of bromoacetic acid in Salmonella typhirurium strains TA 100 and TA 98. Few other recognized genotoxicity assays have been performed and no carcinogenicity studies are available. In the absence of such studies and recognizing the genotoxic potential of bromoacetic

acid, it is not unreasonable to assess the risk of inadvertant bromoacetic acid exposure from a a threshold exposure of 1.5 µg/day (approximately 0.02 µg/kg bw/day) i.e. the threshold of regulation at the FDA for food-contact materials. The estimated maximal daily intake of bromoacetic acid from the use of the FCS is more than two orders of magnitude lower than this threshold dose of 1.5 µg/day. There should thus be no concern from a health perspective with the estimated exposure to bromoacetic acid.

9.2 Study summaries

9.2.1 Genetic toxicity studies

Giller et al. (1997) examined the genotoxicity of bromoacetic acid (MBA) in the SOS chromotest in *Escherichia coli* PQ37, the Ames fluctuation assay utilizing S*almonella typhimurium* strain TA100 and the newt micronucleus assay in larvae at stage 53 of the developmental table. MBA was negative in the SOS chromotest at levels as high as 1000 µg/ml and in the newt micronucleus assay. However, it was active at concentrations as low as 20 µg/ml in the Ames fluctuation assay with S9 fraction added to the incubation medium.

MBA was also found to be positive in the Ames preincubation Salmonella Assay without a metabolic assay system in strain TA100 but negative with a metabolic system and also negative in strains TA98, 1535, 1537 and the E.coli strain WP2UVRA (http://toxnet.nlm.nih.gov/cgi-bin/sis/search).

Another Ames study with a preincubation protocol confirmed the postive responses in strains TA98 and TA100 with and without a metabolic activation system (Kargalioglu et al., 2002).

No further genotoxicity studies have been identified; this also apply to carcinogenicity studies.

9.2.2 In vivo toxicity studies

Linder et al., (1994) found that the oral LD_{50} for MBA was 177 mg/kg of body weight in adult male Sprague-Dawley rats. A summary of the acute toxicity information submitted to the EPA suggested that the acute oral toxicity in the rat is high based on an LD50 value of < 50 mg/kg. Another study with male rats indicated an LD50 of 88 mg/kg (http://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearchView/2E9451CFFD4D64718525693100164732).

MBA did not affect parameters related to male reproductive function when administered to rats as a single dose of 100 mg/kg of body weight or at 25 mg/kg of body weight per day administered repeatedly for 14 consecutive days (Linder et al., 1994a).

In a study on the developmental effects of MBA in Long Evans rats, animals were treated by oral intubation on gestation days 6-15 (plug = 0) with 0, 25, 50 or 100 mg/kg/day (Randal et al., 1991). Maternal observations included clinical signs, weight change and gross evaluation of organs and uterine contents at necropsy (day 20). Live fetuses were examined for external skeletal and soft tissue malformations. Maternal weight increase was reduced and one female died during treatment at the highest dose. Reproductive performance was comparable across groups. The mean percent of resorbed implants per litter was not different from the controls at any dose. Live fetuses were significantly smaller at 100 mg/kg/day. The mean frequency of soft tissue malformations was significantly increased at the high dose. The defects were principally cardiovascular and craniofacial. These results suggest that MBA is developmentally toxic at 100 mg/kg/day.

9.3 NOEL's

The NOAEL for developmental toxicity in rats was determined to 50 mg/kg (Randal et al., 1991).

9.4 Risk Assessment

Based on the NOAEL for developmental toxicity in the rat and an uncertainty factor of 1000, an oral reference dose (RfD) of 50 µg/kg is obtained. Since this RfD is more than 5 orders of magnitude higher than the estimated maximal daily exposure to bromoacetic from the use of the FCS there should be no non-carcinogenic health concern with such exposure.

In the absence of any in vivo genotoxicity studies and any carcinogenicity studies with MBA but recognizing its in vitro genotoxic potential, it is considered appropriate to assess the risk of inadvertant bromoacetic acid exposure related to the threshold exposure of 1.5 µg/day (approximately 0.02 µg/kg/day). Since the estimated maximal daily intake of bromoacetic acid from the use of the FCS is more than two orders of magnitude lower than this threshold there should be no concern from an overall health perspective with the estimated exposure to bromoacetic acid.

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