Detailed comments on DOT CLH Report

Reproductive Toxicity

p12 Part A Section 2.2 Short summary of the scientific justification for the CLH proposal

"The proposed classification and labeling of disodium octaborate tetrahydrate for reproductive toxicity is based on read-across from other tested borates (e.g. boric acid) and borate salts (borax or disodium tetraborate decahydrate) because its hydrolysis results in the formation of the same substances. The resulting classification is comparable to that of the other borates in Annex VI."

Comments:

Whilst we agree with the statement in the CLH report that read across to other borates e.g. boric acid is relevant, the classification proposed should also adequately consider a weight of evidence approach as described in the CLP Regulation (1272/2008/EC) classification criteria in Annex 1, Sections 1.1.1 and 3.7.2.3.

Boric acid shows adverse effects on fertility as well as developmental toxicity in laboratory animals and therefore a classification as reproductive toxicant is warranted. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 under the CLP Regulation may be more appropriate than the proposed Category 1B.

An in depth evaluation of numerous independent epidemiology, worker exposure and mechanistic studies completed since the classification of boric acid and borate salts in 2008 raises doubt about the relevance in humans of the developmental and reproductive effects of boric acid observed in laboratory animals.

The classification for boric acid was carried into the 1st ATP to the CLP regulation from 30th ATP Council Directive 67/548/EEC. In recital 2 of the 30th ATP, reference is made to the study in China: *"The classification and labelling of the substances listed in this Directive should be reviewed if new scientific knowledge becomes available. In this respect, considering recent preliminary, partial and not peer-reviewed information submitted by industry, special attention should be paid to further results of epidemiological studies on the Borates concerned by this Directive including the ongoing study conducted in China …"*

Since publication of the 30th ATP two studies of boron industry workers in China and Turkey chronically exposed to very high levels of borates have been completed and published in peer reviewed publications showing no fertility effects in males (Robbins et al. 2010, Scialli et al. 2010, Duydu et al. 2011). The Chinese and Turkish semen studies in highly exposed workers are a major source of information as to human reproductive toxicity. Not only are these the most exposed workers with exposures measured directly from food, drink and inhalation, the Chinese and Turkish workers studies are the most sensitive studies that have been carried out as semen analysis was performed, a very sensitive detection system for testicular damage. These studies have been discussed briefly in the CLH report for disodium octaborate. The worker studies clearly demonstrate that even under these extreme exposure conditions, the exposures were not high enough to be in the range of dose levels where effects were seen in animals and therefore does not pose a hazard to humans. These worker populations in China and Turkey were selected for investigation because of the known extreme boron exposure conditions not seen elsewhere.

In addition to the studies in boron industry workers, no fertility effects were seen in a population in Chile exposure to high concentrations of boron in drinking water (Barr et al. 1993; Espinoza-Navarro et al. 2010; Cortes et al. 2011).

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Mechanistic data show that the action of boric acid on HDACi and Hox genes occurs at a high dose (1000 mg boric acid/kg bw) and during a very narrow window of gestation (GD 8,9) in laboratory animals. These effects are not likely to be relevant to humans, since the dose of 1000 mg/kg bw in humans would be about 60 - 70 g of boric acid which is more than twice the lethal dose in humans. The mechanism of boric acid is similar to aspirin (action as HDACi and on *Hox* genes), a widely used drug known to cause developmental effects in rodents but not shown to cause teratogenic effects in humans in controlled studies (Di Renzo et al. 2007, 2008; Wery et al. 2003; Narotsky et al. 2004).

A key difference between humans and laboratory animals relative to boric acid toxicity is the large zinc stores in humans compared to laboratory animals. Zinc has been shown to protect against testicular toxicity of chromium, cobalt and cadmium and developmental toxicity of cadmium. Zinc has also been shown to protect against developmental and fertility effects of boric acid in animal and *in vitro* studies including: low acute toxicity of zinc borate, increased fertility NOAEL in 28-day toxicity studies of zinc borate, increased NOAEL for developmental effects (reduced fetal body weights) in a zinc borate developmental dose range finder study, a dose dependent reduction in the boric acid inhibition of differentiation of embryonic stem cells in an embryonic stem cell test, and absence of effects on spermatogenesis in the presence of zinc in boric acid treated cultures of seminiferous tubules. The protective effect of the large zinc stores in the human body against boric acid associated toxicity explains in part the absence of effects in humans exposed to high levels of boron (Daniels 1969; Wragg et al. 1996; Kirkpatrick 2013; Edwards 2013; Hofman-Huther 2013; Martin 2013) .

Therefore, based on a total weight of evidence, Repr. Category 2 H361d: suspected of damaging the unborn child, is considered the appropriate classification. Extensive evaluations of sperm parameters in highly exposed workers have demonstrated no effects on male fertility justifying no classification for fertility. No developmental effects have been seen in highly exposed populations. However, epidemiological studies of developmental effects are not as robust as the fertility studies, warranting the Category 2 H361d. This classification accommodates for both the positive findings in laboratory animals and the absence of effects in humans.

p21 Part B Section 4 HUMAN HEALTH HAZARD ASSESSMENT

"The summaries included in this proposal on disodium octaborate tetrahydrate are predominantly copied from the draft Competent Authority Report and Proposed Decision (CAR, 2006) of the Netherlands prepared in the context of the possible inclusion of the active substance disodium octaborate tetrahydrate in Annex I of Council Directive 98/8/EEC (CAR, June 2006; rev June 2008)."

"The classification of borates for reprotoxicity was also discussed in the Commission Working Group of Specialized Experts in the field of Reprotoxicity in October 2004 (Summary record ECBI/132/04 Rev. 2)."

Comments:

New scientific information has become available since CAR 2006 and Commission Working Group of Specialized Experts (SE) October 2004 meeting occurred, as recognized by the 30th ATP Recital 2, which includes the studies of Chinese and Turkish boron industry workers and the non-occupational exposure study in Chile. Although the CLH report discusses this information, some important details have not been mentioned. Furthermore the studies on the Chinese and Turkish workers have not been considered as part of a total weight of evidence, since the justification in Part B Section 2.2 (p12) and the conclusion in Part B Section 4.11.6 (p54) refers to the current classification of boric acid and disodium tetraborate decahydrate, for which the decision was made before this evidence was available. The various data are provided below, as well as other new evidence to be taken into account.

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- Boron has no effect on Prostate Specific Antigen (PSA) levels

  Two studies of PSA levels in populations chronically exposed to boron show no effect on PSA levels. A study in Turkey was conducted to investigate the reproductive effects of chronic boron exposure in workers employed in a boric acid production plant in Turkey. In order to characterize the external and internal boron exposures, boron was determined in biological samples (blood, urine, semen), in workplace air, in food, and in water sources. Sperm concentration parameters, motility parameters of sperm cells, sperm morphology parameters, DNA integrity with COMET assay, hormone levels (FSH, LH, total testosterone) and total PSA was determined in workers. The PSA level was not statistically significantly different when groups are compared. The mean calculated daily boron exposure of the highly exposed group was 14.45 ± 6.57 (3.32–35.62) mg/day (Duydu et al. 2011).

  In a community-based study of a population exposed to high levels of boron, no statistically significant differences in PSA levels were seen in highly exposed men compared to the control group (Muezzinoglu et al. 2011).

  The SE referred to reduction of PSA by boric acid stating: *"It is used as a marker for leakage from the prostate and as such a marker for prostate cancer. That means that boron interacts with an androgen dependent function of the human prostate, which would be of concern for human fertility"* (Part B Section 8 Annex I p69). However, the information given above allows for a different conclusion to be made and in addition, no reference study was provided by the SE regarding the effect of reduction of PSA by boric acid, so the basis for their statement is unclear.

- Extensive worker studies provide human data on boron levels in blood, semen and urine previously unavailable.

  Since the SE meeting, two extensive worker studies have been conducted that evaluated external exposures, internal boron levels in the blood, semen and urine boron levels, correlated with individual sperm parameters measurements. The external exposures and blood, urine, semen and target tissue boron levels have been correlated with laboratory animal data. A comparison of blood, semen and target organ boron levels in studies of laboratory animals and human studies shows that boron industry worker exposures are lower than untreated control rats. Background boron levels in standard rat chow are high (10-20 ppm), as a result control rats in toxicity studies receive 45 times more boron than background exposure in humans. Blood boron levels in pregnant female control rats is about 0.23 µg B/g (Price et al. 1997), approximately equal to the blood levels in boron industry workers in China, Turkey and U.S. of 0.25, 0.22 and 0.26 µg B/g, respectively (Scialli et al. 2010; Culver et al. 1994; Duydu et al. 2011). (Note: during pregnancy the glomerular filtration rate is increased resulting in increased boric acid excretion and lower blood boron levels than in similarly exposed males or non-pregnant females.) Plasma and seminal vesicle fluid (the major component of semen) boron levels in *untreated* male control rats were 1.94 and 2.05 µg B/g, respectively, while boron levels in testes in rats dosed at the rat fertility LOAEL (26 mg B/kg) was 5.6 µg B/g (Ku et al. 1991,1993). Values in male *untreated control* rats were higher than corresponding boron levels in the highest exposed Chinese boron industry workers with blood boron levels of 1.56 µg B/g and 1.84 µg B/g in semen (Scialli et al. 2010). Blood and semen boron levels in highly exposed Turkish boron workers were also lower than *untreated control* rats with levels of 0.22 and 1.88 µg B/g, respectively (Duydu et al. 2011). Boron levels in testes of rats dosed at the rat fertility LOAEL was over 3x the blood boron levels in highest exposure group of Chinese boron industry workers. The blood level at the lowest animal LOAEL for developmental toxicity (13 mg B/kg) was 1.53 µg B/g, about 6 times greater than typical boron industry workers (Price et al. 1997). No adverse effects on sperm were seen in Turkish boron industry workers or in the most highly exposed subgroup of Chinese boron industry workers drinking boron

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contaminated water at the mine site (mean blood level 1.56 µg B/g). Only under extreme exposure conditions do human levels reach those of this animal LOAEL: the subgroup of Chinese boron workers who also drank contaminated water at the mine site. The highest recorded intake of boron in the Chinese worker study population (7.8mg B/kg bw/day) was almost 50% of the NOAEL for male reproductive toxicity in rats (Scialli et al. 2010). The 16 men who worked at the Pengxiang processing plant did not demonstrate decrements in semen quality in spite of a mean estimated boron intake of 125 mg/day, more than nine times higher than that of other boron workers and almost 100 times higher than the boron intake of remote background controls.

In the SE summary record (Part B Section 8 Annex I p70-71), various statements were made regarding a lack of knowledge on certain key areas. For example The SE noted that no information was available on internal exposure levels, and if a man was accidently heavily exposed then sperm would be collected and one would expect to see an adverse effect. The SE stated not enough human data to disregard the results from animal experiments. SE stated that no knowledge on effective dose in man leading to uncertainty and nothing was known about blood plasma concentrations. As shown above, the SE comments have been addressed with data in humans that provide a better estimate than laboratory animals of the potential effects of borates in sensitive populations. Workers in boron mining and processing industries represent the maximum possible human exposure however their blood and semen boron levels are less than levels in *untreated* control rats. This provides an explanation in part why studies of highly exposed boron industry workers have shown no adverse effects and demonstrates that maximal possible exposures in humans are insufficient to cause reproductive toxicity effects.

- Human data available that demonstrate absence of adverse fertility effects.

Worker exposure studies in Turkey and China and non-occupational exposure group in Chile have been studied. The Chinese and Turkish semen studies in workers highly exposed to boron are a major source of information as to human reproductive toxicity. Not only are these the most exposed workers and the most highly exposed population with exposures measured directly from food, drink and inhalation, but the Chinese and Turkish workers studies are the most sensitive studies that have been carried out as semen analysis was performed, a very sensitive detection system for testicular damage.

Epidemiology studies often lack sufficient statistical power to detect differences that are meaningful. Scialli et al. (2010) performed power calculations of the Chinese worker study and calculated the detection of a 25% difference in sperm concentration between groups at the 5% significance level with 80% statistical power. Moreover, the authors concluded that the study power to detect a doubling of risk of failure to meet WHO criteria for normal semen analysis at the 5% significance level is also about 80%. Altogether, the authors concluded that the statistical power of the study was adequate.

In addition to the absence of effects on male fertility in humans, there is no evidence of developmental effects in humans attributable to boron in studies of populations with high exposures to boron. Epidemiological studies of human developmental effects have shown an absence of effects in exposed borate workers and populations living in areas with high environmental levels of boron. Although the developmental epidemiological studies have methodological deficiencies, collectively these studies consistently show an absence of effects in highly exposed populations (Tuccar et al. 1998; Cöl et al. 2000; Chang et al. 2006; Scialli et al. 2010).

To support classification, the SE concluded that epidemiological studies in humans were insufficient to demonstrate the absence of adverse effect on fertility (Part B Section 8 Annex I p70-71). However this statement was made prior to the studies mentioned above being completed and published.

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- Toxicodynamics evidence identifies differences between animals and humans.

  Since the SE group meeting, a difference in toxicodynamics between laboratory animals and humans has been identified. The protective effect of the large zinc stores in the human body against boric acid associated toxicity explains in part the absence of effects in humans exposed to high levels of boron. Zinc has been shown to protect against testicular toxicity of chromium, cobalt and cadmium (Afonne et al. 2002; Anderson et al. 1993), and developmental toxicity of cadmium (Ahokas et al. 1980; Daston 1982; Fernandez et al. 2003; Hartsfield et al. 1992).

  A similar interaction with boron may well explain the absence of fertility and developmental effects in humans. Protective effect of zinc against boric acid related fertility and developmental toxicity has been shown in several toxicity studies of zinc borate (Daniels 1969; Wragg et al. 1996; Kirkpatrick 2013; Edwards 2013; Hofman-Huther 2013). Details of these studies are discussed in detail under section 4.11.3.

  Normal levels of zinc in soft tissues in humans are over two times greater than in comparative tissues in laboratory animals (Figure 3) (King et al. 2000; Ranjan et al. 2011; Yamaguchi et al. 1996; Florianczyk 2000). These excess zinc stores compared to laboratory animals provide added protection against the toxic effects of exposure to high levels of boric acid not available in laboratory animals by interacting with boric acid to reduce its toxic effects. The high zinc concentrations in humans compared to laboratory animals is also found in the target organs of boric acid, including fetal tissue, epididymis, and testes (Figure 3) (Ahokas et al. 1980; Dorea et al. 1987; Suescun et al. 1981).

  Studies by Di Renzo et al. (2007, 2008) Wery et al. (2003) and Narotsky et al. (2004) show that the boric acid inhibition of HDAC and effect on the *hox* gene occurs at a relative high dose (1000 mg boric acid/kg bw) and during a very narrow window of gestation (GD 8 – 9). Since the dose of 1000 mg/kg bw in humans would be about 60-70 g of boric acid which is more than twice the lethal dose in humans, these effects are not likely relevant to humans.

  Also see comments to Section 4.11.3.

  To support classification the SE concluded that: It is not known whether there are significant differences in the dynamics between humans and laboratory animal models and in the absence of such knowledge it must be assumed that the effects seen in animals could occur in humans. On the basis of kinetic and dynamic considerations it is assumed that the animal data are relevant to humans.(Part B Section 8 Annex I p72). However, the studies showing the protective effect of zinc in humans brings these assumptions into question.

- Human exposure data strongly indicate the high exposure levels in animal studies cannot be reached

  Workers in boron mining and processing industries represent the maximum possible human exposure. Boron concentrations were determined in biological samples (blood, urine, semen), in workplace air, in food, and in water sources. Exposures of these workers included high levels of borate dust and drinking water contaminated with boron. Daily exposures are shown in Table 1 and a comparison of exposures to fertility and developmental NOAELs are presented in Figure 1. While under very extreme exposure conditions (high inhalation exposures and high oral exposures via contaminated drinking water at the mining site), no fertility effects were seen in workers.

Table 1: Comparison Exposure Values

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China	Turkey
Daily Boron Exposure (mean ± SD, range)	
42 ± 58 mg B/day	14.45 ± 6.57 mg B/day
125 ± 89 mg B/day*	
Urine Boron Levels	
14.7 mg B/g creatinine (range 1.46-117)	9.8 ± 5.1 mg B/g creatinine (range 1.15 – 32.68)
63.3 ± 43 mg B/g creatinine*	
Blood Boron Levels	
252 ng B/ml (range 22-2482)	224 ± 69 ng B/g (range <LOQ – 454.02)
1558 ± 1041 ng B/ml*	
Semen Boron Levels	
592 ng B/ml (range 59.7 – 4087)	1876 ± 2255 ng B/g
1844 ng B/ml*	

*Pengxiang plant

This information (available after the SE meeting) therefore refutes the SE conclusions that: *"potential human exposure levels via inhalation and oral routes could be within one order of magnitude of the NOELs for reproductive toxicity found in animal studies. The threshold level for effects in humans is not known but it cannot be excluded that it could be below the level causing vomiting in humans."* (Part B Section 8 Annex I p72).

p38 Part B Section 4.7.1.5 Human information - *Human Data from Poison Control Centres and Literature Cases*

Comments:

References made of boric acid poisoning case reports need to be clarified since they are misleading as presented in the CLH report.

The anemia observed in the case report of a 28 year old consuming 500 mg/day of boric acid was unlikely to have been caused by the amount of boric acid ingested, rather due to the malnutrition associated with pica behavior from

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ingesting large amounts of talc in the place of a nutritious diet. Calculating the 0.5 g of boric acid per day equates to 8.3 mg boric acid/kg/day or 1.46 mg B/kg per day, well below a dose shown to cause anemia. For this reason this case report should not be relied upon since the anemia was likely caused by malnutrition and not by boric acid.

In any event, the cases referenced in the CLH report do not reflect today's situation: cases were a long time ago and reflect out of date practices. Much of the data comes from the mid-1800s to around 1940, when boric acid and disodium tetraborate decahydrate were used systematically for a variety of medical conditions including amenorrhea, malaria, epilepsy, urinary tract infection and exudative pleuritis (Kliegel, 1980).

p39 Part B Section 4.7.1.7 Summary and discussion of repeated dose toxicity

"The dogs appeared to be more sensitive to the effects of boric acid on the testes. Similar results were obtained from studies with disodium tetraborate decyhadrate. The 90 days feeding study with boric acid in dogs yielded an overall NOAEL (2.6 mg/kg bw, equal to 0.46 mg B/kg bw/day), based on reduced testes weight and histological changes in the testes at a dose of 24 mg/kg bw/day, (4.2 mgB/kg bw/day). This finding is supported by the study with disodium tetraborate decahydrate, in which a (statistically non-significant) reduction in testicular weight and histological changes in the testes were observed at 42 mg/kg bw/day (4.7 mg B/kg bw/day) and severe testicular atrophy at 341 mg/kg bw/day (38 mg B/kg bw/day)."

Comments:

We agree with the conclusion of the CLH report on the quality of the dog studies noted in section 4.11.1.1 that the 90 day dog studies on boric acid and disodium tetraborate decahydrate (Paynter, 1963a,b) are of limited value and considered inadequate for risk assessment although they support qualitatively that the main target organ of boron toxicity is the testis in laboratory animals.

4.11.1.1 "*In a 90-day and a 2-year oral toxicity studies with boric acid in dogs the testes were identified as a major target for boron treatment. However, these studies were considered not acceptable for quantitative evaluation for the biocide regulation (Paynter 1963a, and an unknown, as summarised in CAR 2006).*"

"*In a 90-day and a 2-year oral toxicity studies with disodium tetraborate decahydrate in dogs again the testes were identified as a major target for boron treatment. However, these studies were considered not acceptable (Paynter 1963b, and unknown, as summarised in CAR 2006).*"

The limitations and flaws of the dog studies are described in more detail in Annex 1 of the EU RAR (2007).

p49 Part B Section 4.11.1.2 Toxicity for reproduction, Human information

"It is noted that these exposure levels and the exposure assessed in the Bandirma boric acid production plant (Duydu, 2012) are considerably lower than exposures, which have previously led to reproductive effects in experimental animals."

Comments:

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The Chinese and Turkish semen studies in highly exposed workers are a major source of information as to human reproductive toxicity. These are the most exposed workers with exposures measured directly from food, drink and inhalation. The Chinese and Turkish workers studies are the most sensitive studies that have been carried out as semen analysis was performed, a very sensitive detection system for testicular damage.

Occupational exposures in the studies in Chinese, Turkish and USA workers were lower than laboratory exposures of animals, but the highest of these describe the upper limits of exposures in production of boron-containing products. Because workers reported eating in contaminated areas, the Chinese exposures were higher than would be expected from production processes.

Highest reported non-occupational exposures (Barr et al. 1993; Cortes et al. 2011) have not approached the mean exposure of 42 mg B/day found in the Chinese workers (main study group). Although the studies of boron industry workers in China and Turkey are the highest documented occupational exposures and the highest exposed populations, the mean exposure levels of the main study cohort are only about one third to one quarter of the NOAEL for testis effects in rodents. The highest recorded intake of boron in the Chinese worker study population (7.8mg B/kg bw/day) was almost 50% of the NOAEL for male reproductive toxicity in rats (Scialli et al. 2010). The 16 men who worked at the Pengxiang processing plant did not demonstrate decrements in semen quality in spite of a mean estimated boron intake of 125 mg/day, more than nine times higher than that of other boron workers and almost 100 times higher than the boron intake of remote background controls. These studies show that humans are not more sensitive to this type of toxic effect than rodents. This can be seen when comparing the NOAEL for fertility effects in the rat and the human NOAEL based on highest occupational exposures in the Chinese workers study. The NOAEL in rats is 17.5 mg B/kg/day; divided by the human NOAEL of at least 2.08 mg B/kg day (based on 60 kg person) results in a ratio of only 8.75, over 10 times lower than the default uncertainty factor of 100 often used in risk assessments.

Workers in boron mining and processing industries represent the maximum possible human exposure, however a comparison of blood and target organ boron levels in studies of laboratory animals and human studies was evaluated showing that boron industry worker exposures are lower than *untreated control* rats. No adverse effects on sperm were seen in the highest exposed groups of boron industry workers. This explains why highly exposed boron industry workers have shown no adverse effects and demonstrates that maximal exposures in humans are insufficient to cause reproductive toxicity effects.

The highest non-occupational exposure found were populations in Northern Chile in which estimated intake of boron was 21 to 27 mg B/day, which correlated to naturally high boron concentrations in local rivers (Barr et al. 1993). Exposure levels of boron in drinking water and urine was measured from volunteers in Arica, an area in the North of Chile with high levels of naturally occurring boron (Cortes et al. 2011). The concentration of boron in urine varied between 0.45 and 17.4 mg/L, with a median of 4.28 mg/L and was found to be correlated with tap water sampled from the homes of the volunteers (r=0.64). Espinoza-Navaro et al. analyzed sperm for total sperm count, sperm concentration, volume, vitality, pH, morphology, overall motility and grade A motility in a sample of 102 healthy young males aged 18 to 30 years residing in Arica, Chile. The volunteers also completed a questionnaire about fertility, habits and andrologic diseases. Males sampled in Arica had normal sperm values in comparison with international reports (Espinoza-Navarro et al. 2010).

Comparison of blood and tissue boron levels in humans and rats

A comparison of blood, semen and target organ boron levels in studies of laboratory animals and human studies shows that boron industry worker exposures are lower than untreated control rats. Background boron levels in standard rat chow are high (10-20 ppm), as a result control rats in toxicity studies receive 45 times more boron than background exposure in humans (See Figure 1). Blood boron levels in pregnant female *untreated control* rats is

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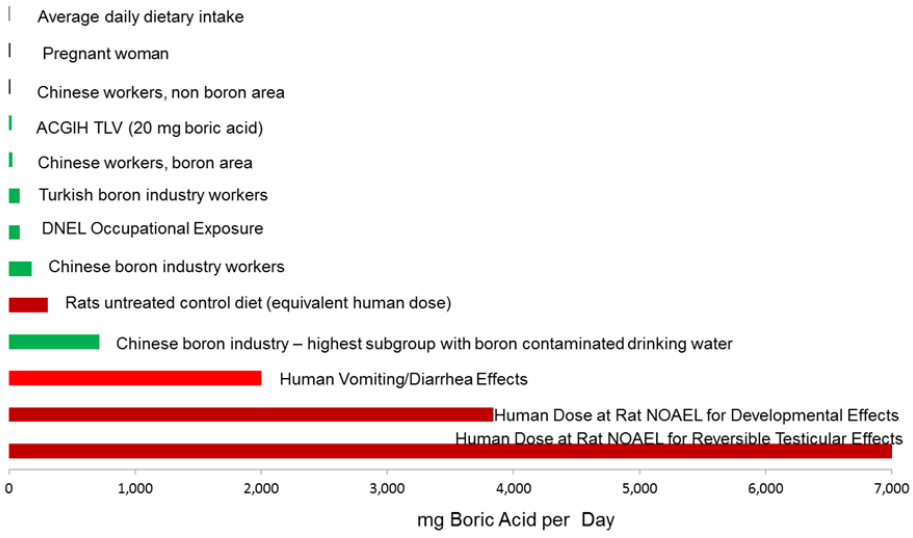
about 0.23 µg B/g (Price et al. 1997), approximately equal to the blood levels in boron industry workers in China, Turkey and U.S. of 0.25, 0.22 and 0.26 µg B/g, respectively (Scialli et al. 2010; Duydu et al. 2011; Culver et al. 1994). (The glomerular filtration rate is increased during pregnancy resulting in increased boric acid excretion and lower blood boron levels than in similarly exposed males or non-pregnant females.) Plasma and seminal vesicle fluid (the major component of semen) boron levels in untreated male control rats were 1.94 and 2.05 µg B/g, respectively, while boron levels in testes in rats dosed at the rat fertility LOAEL (26 mg B/kg) was 5.6 µg B/g (Ku et al. 1991,1993). Values in male control rats were higher than corresponding boron levels in the highest exposed Chinese boron industry workers with blood boron levels of 1.56 µg B/g and 1.84 µg B/g in semen (Scialli et al. 2010). Blood and semen boron levels in highly exposed Turkish boron workers were also lower than control rats with levels of 0.22 and 1.88 µg B/g, respectively (Duydu et al. 2011). Boron levels in testes of rats dosed at the rat fertility LOAEL was over 3x the blood boron levels in highest exposure group of Chinese boron industry workers.

The blood level at the lowest animal LOAEL (13 mg B/kg developmental toxicity) was 1.53 µg B/g, about 6 times greater than typical boron industry workers (Price et al. 1997). No adverse effects on sperm were seen in Turkish boron industry workers or in the most highly exposed subgroup of Chinese boron industry workers drinking boron contaminated water at the mine site (mean blood level 1.56 µg B/g,). Since no boron accumulation occurs in soft tissues (testes) over plasma levels, biological monitoring in humans provides direct comparison to test animal target organ boron levels.

Figure 1. Rat and Human Exposure to Boric Acid



p52 Part B Section 4.11.3 Toxicity for reproduction, Other relevant information

"This indicates that the described mechanism [*hox*, hdaci] *is likely to be also relevant for humans."*

Comments:

The CLH report briefly discusses the mechanisms of boric acid related effects; histone deacetylase inhibition (HDACi) and effects on *hox* genes. While HDACi and *hox* genes are present in humans, the high doses of boric acid that are required and the narrow exposure period during gestation shown to cause the HDACi effect and the

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effects on *hox* genes in laboratory animals makes the likelihood of similar effects in humans low. These mechanisms are discussed in more detail below:

- ***Histone Deacetylase Inhibitors (HDACi)***

  The ability of some histone deacetylase inhibitors (HDACi) to induce hyperacetylation on mouse embryo tissues has been correlated to teratogenic properties in mice. Hyperacetylation of target organs, such as the embryonic axial structures (somites), has been directly related to axial skeletal malformations (fusions of vertebrae and/or ribs, duplication of axial segments, homeotic transformations) in mice. The targets of HDACi in embryos are the histone core proteins of somites. Somites are transient embryonic structures giving rise to dermal, muscular, and skeletal structures of the trunk (Di Renzo et al. 2008).

  As noted in the CLH report, recent studies by Di Renzo et al. (2007) provide evidence for HDAC inhibition by boric acid that suggests a molecular mechanism for the induction of boric acid related malformations in laboratory animals. The data indicate that boric acid is able to induce hyperacetylation in specific embryonic target tissues (somites), associated with direct interference with HDAC enzyme activity. No effects were recorded at the level of other embryonic tissues and at the level of other proteins. HDAC inhibition could be the mechanism for hyperacetylation of somites and consequently for axial malformations observed at term of gestation in laboratory animals (Di Renzo et al. 2007). Since the critical period for the effects of boric acid on development of the axial skeleton in laboratory animals is very narrow and requires a high dose during that period (1000 mg boric acid/kg on day 8 of gestation), the likelihood of similar effects in humans is low since the dose of 1000 mg/kg bw in humans would be about 60-70 g of boric acid which is more than twice the lethal dose in humans.

  <u>Similarity of aspirin and boric acid developmental effects in laboratory animals</u>

  Inhibition of histone deacetylases (HDACi) by sodium salicylate (SAL), the natural deacetylated form of aspirin, has also been shown as a mechanism of teratogenesis (axial skeletal malformations) in laboratory animals (Di Renzo et al. 2007, 2008). Similarly to boric acid, SAL is reported to induce axial abnormalities when administered to pregnant rodents. In particular, supernumerary ribs (lumbar ribs) have been associated with the oral administration of 300 mg/kg SAL to pregnant mice (gestation day 8). The anti-inflammatory properties of salicylates have been known for decades, and they are among the oldest and most widely used drugs in the world. SAL is responsible for the anti-inflammatory action of aspirin, introduced into clinical practice more than 100 years ago and one of the most widely used medicines. Their low toxicity in adults is well known. Although known to cause developmental effects in laboratory animals, there is no evidence that moderate therapeutic doses of salicylates cause fetal damage in human beings. Controlled human studies of SAL and aspirin have not demonstrated teratogenicity (HSDB 2012). Salicylates have been recently evaluated for new multiple therapeutic activities, including range of antineoplastic properties of both synthetic salicylates and a vegetable-rich diet (Di Renzo et al. 2008).

  When aspirin is administered during the third trimester there is an increase in perinatal mortality, anemia, antepartum and postpartum hemorrhage, prolonged gestation, and complicated deliveries; thus, the recommendation against its use during this period (Hardman et al. 2006). During the last weeks of gestation, long-term high-dose salicylate therapy may cause prolonged gestation, increased risk of postmaturity and fetal neonatal hemorrhage. The effects from exposure during the third trimester when organ development is complete are unrelated to the teratogenic effects seen in laboratory animals that occur earlier in the gestation during organogenesis (gestation day 8). Aspirin is classified as FDA pregnancy

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category C. Chronic maternal ingestion is associated with an increased incidence of stillbirths, antepartum/postpartum bleeding, prolonged pregnancy/labor, and lower birth weight (HSDB 2012).

The mechanism of developmental effects of aspirin in laboratory animals is relevant to boric acid since both SAL and boric acid act via a similar mechanism in causing developmental effects in mice. Although no large scale studies of boric acid have been conducted in humans, large populations routinely use aspirin for extended periods of time and are the most widely used drug worldwide. While developmental effects from aspirin have been observed in laboratory animals, similar effects have not been seen in humans. This provides supporting evidence that developmental effect in humans from exposure to high levels of boric acid is improbable.

Anti-tumor properties of Boron

HDAC inhibition also explains the mechanism of anti-carcinogenic effects of increased dietary intake of boron. HDACi are known to induce cell growth arrest cell death (apoptosis) and lineage differentiation. Molecules with properties similar to the HDACi show antineoplastic activity. For these reasons HDACi are considered promising molecules in the treatment of neoplastic diseases (Di Renzo et al. 2008).

- ***Boric Acid Effects on Hox Gene Expression***

Boric acid related axial abnormalities in rodents have also been attributed to a shift in Hox gene expressions (Wery et al., 2003, Di Renzo 2008). During embryonic development, positional information determining the craniocaudal identity of the somites, the precursors to the vertebrae, is thought to be conferred by the hox genes. Hox expression in the paraxial mesoderm begins during gastrulation before the formation of the somites. Hox genes have overlapping domains of expression in the somites and prevertebrae (PV) that extend from the caudal end of the embryo to a precise cranial limit that is correlated to the linear order of the genes within a given cluster. This expression pattern along the craniocaudal axis of the embryo suggests a combinatorial code according to which the expression of a given combination of hox genes will specify the identity of a vertebral segment (Narotsky et al. 2004).

Studies by Wery et al. (2003) and Narotsky et al. (2004) suggest an impact of boric acid on the basic control mechanisms that define the positional identity of the somites and, consequently, the vertebrae. Homeotic genes that are known to confer positional information in the cervical and the cranial most thoracic regions include hoxd4, a4, a5, c5, c6, and a6. Narotsky et al. (2004) hypothesized that hox expression may be affected by GD-9 exposure to boric acid. Comparison of expression patterns between boric acid exposed embryos and controls revealed a shift in the expression domain of two genes, hoxc6 and hoxa6 on GD 13.5. These shifts are likely to result in posterior transformation of cervical vertebrae later in development, without altering the total number of vertebrae. This is consistent with the observed morphological defects at GD 21 after boric acid exposure, suggesting that these hox gene alterations are part of the dysmorphogenic cascade resulting from boric acid exposure (Narotsky 2004).

As discuss previously, similar to boric acid, sodium salycilate (SAL) was reported to induce axial abnormalities when administered to pregnant rodents. In particular, supernumerary ribs (lumbar ribs) have been associated with the oral administration of SAL (300 mg/kg) to pregnant rats and have been attributed to an anterior transformation of lumbar vertebrae identity into thoracic vertebrae identity, as suggested by Hox gene expression analysis (Wery et al., 2003; Wery et al., 2005; Di Renzo 2008).

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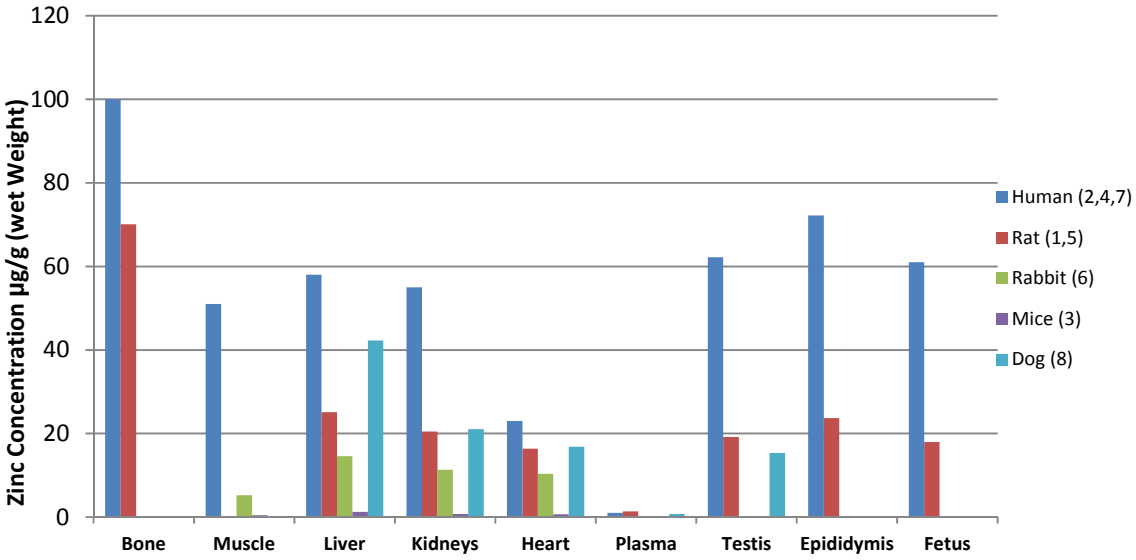
Studies by Wery et al. and Narotsky et al. show that the effect on the hox gene occurs at a relative high dose (1000 mg boric acid/kg) and during a very narrow window of gestation (GD 9).These effects are unlikely to occur in humans since the dose of 1000 mg/kg bw in humans would be about 70 g of boric acid which is more than twice the lethal dose in humans.

- ***Protective Effects of Zinc against Boric Acid Reproductive and Developmental Toxicity***

The protective effect of the large zinc stores in the human body against boric acid associated toxicity explains in part the absence of effects in humans exposed to high levels of boron. Zinc has been shown to protect against testicular toxicity of chromium, cobalt and cadmium (Afonne et al. 2002; Anderson et al. 1993), and developmental toxicity of cadmium (Ahokas et al. 1980; Daston 1982; Fernandez et al. 2003; Hartsfield et al. 1992). A similar interaction with boron may well explain the absence of fertility and developmental effects in humans as demonstrated by reduced boric acid related developmental and fertility effects in studies of zinc borate ((Daniels 1969; Wragg et al. 1996; Kirkpatrick 2013; Edwards 2013; Hofman-Huther 2013) .

Normal levels of zinc in soft tissues in humans are over two times greater than in comparative tissues in laboratory animals (Figure 2) (King et al. 2000; Ranjan et al. 2011; Yamaguchi et al. 1996; Florianczyk 2000). These excess zinc stores compared to laboratory animals provide added protection against the toxic effects of exposure to high levels of boric acid not available in laboratory animals by interacting with boric acid to reduce its toxic effects. The high zinc concentrations in humans compared to laboratory animals is also found in the target organs of boric acid, including fetal tissue, epididymis, and testes (Figure 2) (Ahokas et al. 1980; Dorea et al. 1987; Suescun et al. 1981).

Figure 2: Tissue Levels of Zinc in Humans and Laboratory Animals



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(1) Ahokas et al. 1980; (2) Dorea et al. 1980; (3) Florianczyk 2000; (4) King et al. 2000; (5) Muzzio 2010; (6) Ranjan et al. 2011; (7) Suescun et al. 1981; (8) Yamaguchi et al. 1996

Plasma provides a metabolically active transport compartment for zinc. Zinc is most often complexed to organic ligands (existing in loosely or firmly bound fractions) rather than free in solution as metallic ion. Zinc is found in diffusible or non-diffusible forms in the blood. In the diffusible form, approximately two-thirds of plasma zinc is freely exchangeable and loosely bound to albumin. The diffusible form of zinc also includes zinc bound to amino acids (primarily histidine and cysteine). The zinc-albumin complex is in equilibrium with the zinc-amino acid complex. The zinc-amino acid complex can be transported passively across tissue membranes. The diffusible form of zinc is likely the form available to interact with boric acid. The liver, pancreas, bone, kidney, and muscle are the major tissue storage sites (ATSDR 2005).

The protective effect of zinc against boric acid toxicity is demonstrated by the low acute toxicity of zinc borate compared to disodium tetraborate pentahydrate, both with equivalent boron concentrations, increased fertility NOAEL in 28-day toxicity studies of zinc borate, increased NOAEL for developmental effects (reduced fetal body weights) in developmental dose range finder study, a dose dependent reduction in the boric acid inhibition of differentiation of embryonic stem cells in an embryonic stem cell test, and absence of boric acid related effects on spermatogenesis in the presence of zinc in boric acid treated cultures of seminiferous tubules (Daniels 1969; Wragg et al. 1996; Kirkpatrick 2013; Edwards 2013; Hofman-Huther 2013; Martin 2013).

Zinc borate ($2ZnO{\cdot}3B_2O_3{\cdot}3.5H_2O$) hydrolyzes under high dilution conditions to zinc hydroxide via zinc oxide and boric acid formation. Zinc hydroxide solubility is extremely low under neutral and basic conditions. This leads to a situation where zinc borate hydrolyses to zinc hydroxide and boric acid at neutral pH quicker than it solubilizes. Therefore, it can be assumed that at physiological conditions and neutral and lower pH zinc borate can be considered to be hydrolyzed to boric acid and zinc hydroxide, and subsequently absorbed in the gastrointestinal tract as boric acid and zinc as shown by a toxicokinetic study of zinc borate in rats (Muzzio et al. 2010). However, because of the distinctly different toxicological profile of zinc borate compared to boric acid, read across to boric acid has been shown to be inappropriate for zinc borate (Wragg et al. 1996; Kirkpatrick 2013).

Zinc Borate breaks down as follows:

$$2ZnO{\cdot}3B_2O_3{\cdot}3.5H_2O + 3.5\ H_2O + 4\ H^+ \rightleftharpoons 6\ H_3BO_3 + 2\ Zn^{2+}$$

$$2\ Zn^{2+} + 4\ OH^- \rightleftharpoons 2\ Zn(OH)_2$$

Overall equation

$$2ZnO{\cdot}3B_2O_3{\cdot}3.5H_2O + 7.5\ H_2O \rightleftharpoons 2\ Zn(OH)_2 + 6\ H_3BO_3$$

Zinc Borate Toxicity Studies

Because zinc borate ($2ZnO{\cdot}3B_2O_3{\cdot}3.5H_2O$) hydrolyzes under high dilution conditions to zinc hydroxide via zinc oxide and boric acid formation, subsequently absorbed via the gastrointestinal tract as boric acid and zinc (Muzzio et

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al. 2010), studies of zinc borate provide important data on the protective effects of zinc on boric acid related fertility and developmental toxicity.

Toxicokinetic Study of Zinc Borate

The disassociation of zinc borate and absorption of boric acid and zinc was demonstrated by a toxicokinetic study in rats of zinc borate. Following a single oral dose (1000 mg/kg) of zinc borate, zinc and boron appeared in rat plasma and tissue samples, indicating the hydrolysis of zinc borate in the gastrointestinal tract, and subsequent systemic absorption of zinc and boron (Muzzio et al. 2010). The gastrointestinal tract was the primary elimination route for zinc, while urinary excretion via the kidneys was the primary elimination route for boron.

Zinc Borate Acute Toxicity Study

No mortality occurred in an acute toxicity study of zinc borate in rats administered 10 g/kg-bw, equivalent to 1492 mg B/kg bw (Daniels 1969) compared to disodium tetraborate pentahydrate with a LD50 value of 3.3 g/kg-bw, equivalent to 488 mg B/kg bw. No mortality was observed at a boron dose three times the boron dose shown to produce 50% mortality in the absence of zinc.

28-Day Oral Toxicity Studies of Zinc Borate

No toxic effects were observed in the testes of males administered a similar zinc borate compound in a 28-day repeated dose oral gavage toxicity study, an equivalent dose of 50 mg B/kg bw (Wragg et al. 1996). The LOAEL for testicular effects is 26 mg B/kg body weight. This shows that Zn interacts with boric acid in the body reducing the toxicity of boric acid.

In a 28-day dose range finding study of zinc borate a NOAEL of 37.5 mg B/kg bw and LOAEL of 75 mg B/kg bw for male fertility effects determined by minimal histopathologic findings in testes and epididymides was observed. Since hypospermia was not observed at the LOAEL, the effects observed in the 75 mg B/kg bw group were not considered toxicologically significant (Kirkpatrick 2013).

Dose Range-Finding Prenatal Developmental Toxicity Study of Zinc Borate

Compared to boric acid, a 55% increase in the developmental NOAEL based on lower fetal body weights was demonstrated in a GLP developmental dose range finder study of zinc borate (Edwards 2013). The developmental toxicity NOAEL of zinc borate was 14.92 mg B/kg bw compared to 9.6 mg B/kg bw for boric acid and sodium borates in the absence of zinc.

Embryonic Stem Cell Test

An embryonic stem cell test (EST) was performed to investigate the embryotoxic potential of boric acid in the presence of varying concentrations of zinc *in vitro.* The concentration of boric acid used in this study has been shown in previous EST tests to cause a 50% inhibition of differentiation of D3 cells into contracting myocardial cells (Genschow et al. 2004). No greater sensitivity of embryonic stem cells compared to fully differentiated cells was observed and no concern for *in vivo* embryotoxicity is triggered for boric acid at various concentrations of zinc. A reduction in the boric acid inhibition of differentiation of D3 embryonic stem cells was observed with increasing concentrations of zinc (See Figure 3) (Hofman-Huther 2013).

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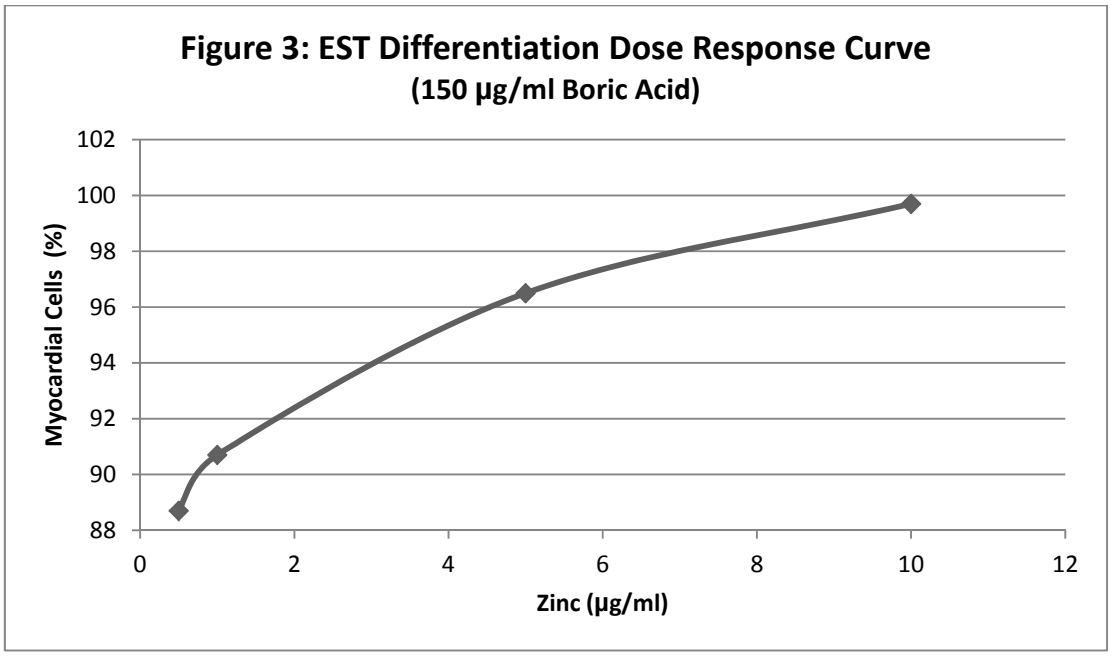

Spermatogenesis Study

An *in vitro* study of the effect of zinc on boric acid related toxicity of spermatogenesis was conducted using primary cultures of seminiferous tubules from rats. Seminiferous tubules were cultured in the presence of 32 ug/ml boric acid and varying concentrations of zinc chloride equivalent to 0.48, 1.2, 2.4 and 4.8 ug Zn/ml to determine the effects on the number of somatic cells (sertoli and myoid cells) and germ cells (pre-meiotic cells (spermatogonia), meiotic cells (young spermatocytes, middle to late pachytene spermatocytes and secondary spermatocytes), and post-meiotic cells (round spermatids)) after 1 and 2 weeks of culture. An absence of boric acid related effects on spermatogenesis was observed in the presence of zinc (See Figures 4-6) (Martin 2013).

A dose dependent increase of somatic cells was observed at day 14 in the presence of zinc (Figure 4). In the presence of boric acid, a decrease in the number of total germ cells was observed. A dose dependent increase in the number of germ cells was observed at day 7 and 14 in the presence of zinc (Figure 5).

Boric acid decreased the number of round spermatids at day 7 and 14 (Figure 6). When zinc is added to the cultures, an increase in the number of round spermatids as compared to the effect of boric acid alone was observed on day 7, and increased the number of round spermatids in a dose dependent manner on day 14. The number of round spermatids is a useful parameter since these cells have completed the two meiotic divisions and the differentiation of round spermatids in elongated spermatids and spermatozoa involves only cytological differentiation, without DNA synthesis (this is an "end-product"). These results suggest that zinc interacts with boric acid reducing its toxicity on the meiotic divisions.

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Figure 4

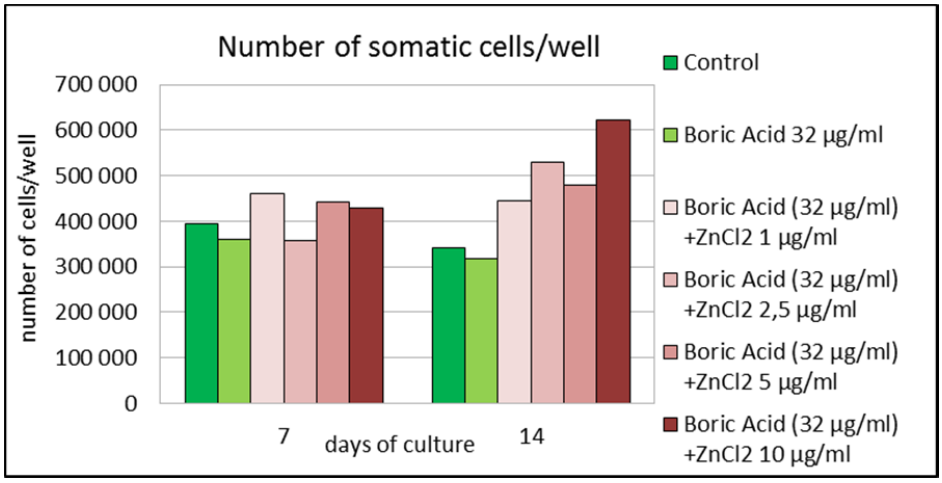

Figure 5

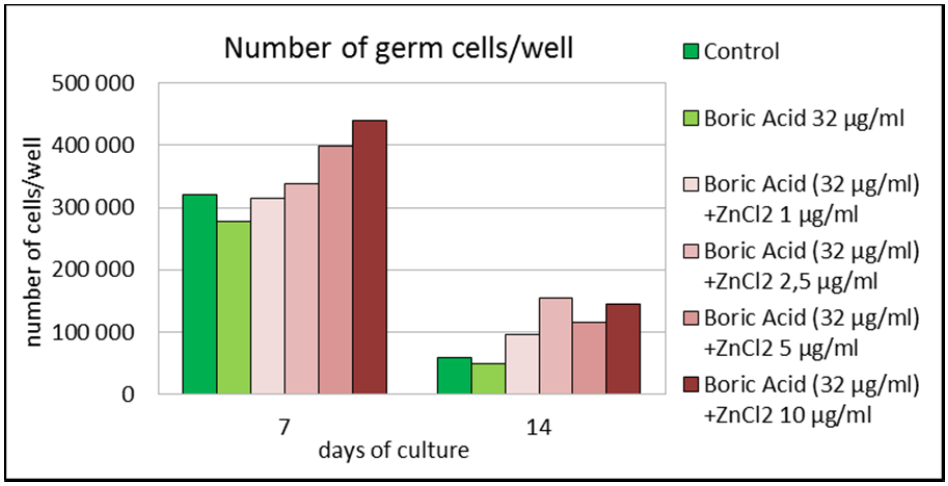

Figure 6

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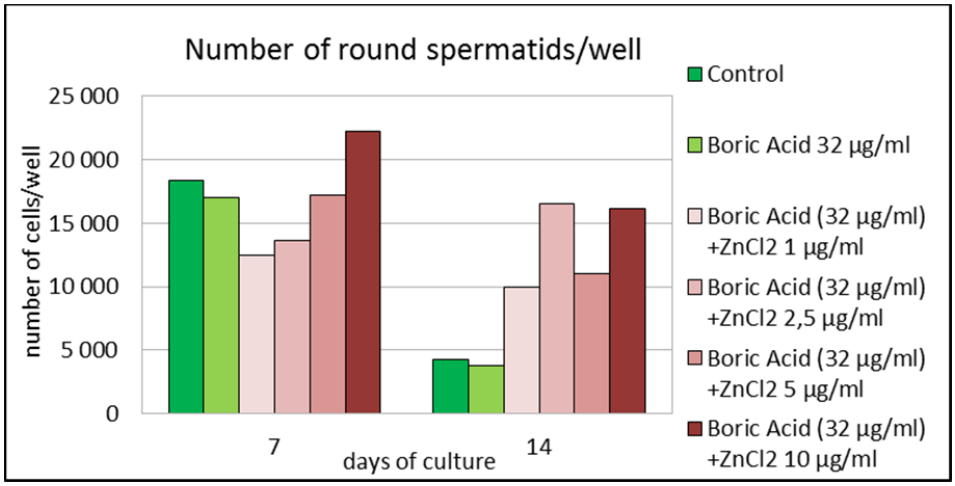

90-Day Oral Toxicity Study of Zinc Borate

A 90-day oral toxicity study of zinc borate is currently underway. Endpoints of the 90-day study include complete spermatogenesis evaluation and microscopic examination of the testes and epididymis which will provide more information on the impact of zinc on boric acid related fertility effects in males.

Zinc Concentration in the Developing Fetus

The protective effects of zinc are present early in the developing human fetus. Significantly higher concentrations of zinc in the human fetus compared to laboratory animals have been reported. Fetuses and neonates have higher concentrations of zinc than adults. The zinc reserves of the human neonate are deposited during fetal development. At the time of birth, a quarter of the body Zn is in the liver. Liver zinc concentration is higher in fetuses and neonates than in adults. Liver zinc does not seem to change even in disease states that lower plasma zinc except in alcoholism. There was a comparable range of zinc values of 10 to 300 ug/g fresh tissue for both pre-term and term babies. The value of 10 ug/g was found only in one 4-month-old infant (Dorea et al. 1987).

The transport of essential trace elements such as zinc from mother to fetus varies throughout gestation with a vigorous mother-to-fetus uphill zinc transfer. Conversely, cord blood zinc is higher than in maternal blood plasma and decreases with gestational age (GA). In addition, the concentration ratio between circulating zinc in the mother and the infant tended to decrease as GA increased. This difference is more marked earlier in the last trimester, than late. Maternal and cord plasma zinc according to gestational age ranged from 24– 28 weeks a maternal/cord zinc ratio 0.51 (± 0.14) increasing to 0.72 (± 0.21) 38– 42 weeks (Perveen et al. 2002).

Studies that are currently ongoing or planned that will provide more information on the role zinc plays in developmental and fertility effects of boric acid include:

- 90-day oral toxicity study of zinc borate (to be completed October 2013),
- Developmental toxicity study of zinc borate (to be completed in 2014).

p53 Part B Section 4.11.5 Toxicity for reproduction, Comparison with criteria

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"The human data does not contradict the animal data. Therefore, there is no evidence that the effects observed in animals are not relevant to humans."

Comments:

The human data contradict the assumption that humans are more sensitive than laboratory animals. At 2.08 mg B/kg/day no fertility effects were observed. When 100 uncertainty factor is applied to the LOAEL of 26 mg B/kg bw, this equates to 0.26 mg B/kg, and using an UF of 60 equates to 0.43 mg B/kg, all well below the exposures in workers without adverse effects. The NOAEL in rats is 17.5 mg B/kg/day; divided by the human NOAEL of at least 2.08 mg B/kg day (based on 60 kg person) results in a ratio of only 8.75. The highest recorded intake of boron in the Chinese worker study population (7.8mg B/kg bw/day) was almost 50% of the NOAEL for male reproductive toxicity in rats (Scialli et al. 2010). The 16 men who worked at the Pengxiang processing plant did not demonstrate decrements in semen quality in spite of a mean estimated boron intake of 125 mg/day, more than nine times higher than that of other boron workers and almost 100 times higher than the boron intake of remote background controls. This data shows that humans are not more sensitive to the adverse reproductive effects of boric acid than laboratory animals.

p54 Part B Section 4.11.6 Toxicity for reproduction, Conclusions on classification and labelling

"Based on the clear adverse developmental and fertility effects of borates in toxicological studies in several animal species it is proposed to classify disodium octaborate tetrahydrate with reproduction category 2 and assign risk phrases R60-61 according to Directive 67/548/EEC.

Based on the adverse developmental and fertility effects of borates in rats and rabbits, disodium octaborate tetrahydrate should be classified with Repr 1B, H360FD May damage fertility. May damage the unborn child according to Regulation EC 1272/2008.

This is in line with the proposal of the EU commission working group of specialized experts in the field of reprotoxicity (see Annex I, Summary record ECBI/132/04 Rev 2, 2004) and the harmonised classification of several other borates."

Comments:

The proposal of the EU Commission Working Group of Specialized Experts and harmonized classification of borates were prior to publication of the results of the worker studies in China and Turkey, and studies in Chile. Further, mechanistic data was not available which is today and submitted during this public consultation. Therefore, these new data should be considered as part of a weight of evidence approach as indicated by the CLP regulation.

The following passages describing the basis for classification of reproductive toxicity are extracted from REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 (emphasis added):

"Category 1B:

Presumed human reproductive toxicant

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other

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toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. **However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate**.

Category 2:

Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects." (Table 3.7.1(a))

"Classification is made on the basis of the appropriate criteria, outlined above, and **an assessment of the total weight of evidence** (see 1.1.1). Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects." (Section 3.7.2.2.1)

1.1.1.3 The role and **application of expert judgement and weight of evidence determination**. **A weight of evidence determination means that all available information bearing on the determination of hazard is considered together**, such as the results of suitable in vitro tests, relevant animal data, information from the application of the category approach (grouping, read-across), (Q)SAR results, **human experience such as occupational data** and data from accident databases, epidemiological and clinical studies and well documented case reports and observations. **The quality and consistency of the data shall be given appropriate weight.** Information on substances or mixtures related to the substance or mixture being classified shall be considered as appropriate, as well as site of action and mechanism or mode of action study results. **Both positive and negative results shall be assembled together in a single weight of evidence determination**."

Weight-of-Evidence Considerations

An in depth evaluation of numerous independent studies raises doubt about the relevance in humans of the developmental and reproductive effects of boric acid observed in laboratory animals:

- No evidence of developmental effects in humans attributable to boron has been observed in studies of populations with high exposures to boron.
- While boron has been shown to adversely affect male reproduction in laboratory animals, studies of highly exposed boron industry workers consistently show no reproductive effects attributable to boron.
- Workers in boron mining and processing industries represent the maximum possible human exposure however a comparison of blood, semen and target organ boron levels in studies of laboratory animals and human studies shows that boron industry worker exposures are lower than untreated control rats.

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- Mechanistic data show that the action of boric acid on HDACi and Hox genes occurs at a high dose (1000 mg boric acid/kg bw) and during a very narrow window of gestation (GD 8,9) in laboratory animals. These effects are not likely to be relevant to humans, since the dose of 1000 mg/kg bw in humans would be about 60-70 g of boric acid which is more than twice the lethal dose in humans.

- The mechanism of boric acid is similar to aspirin (action as HDACi and on *Hox* genes), a widely used drug known to cause developmental effects in rodents but not shown to cause teratogenic effects in humans in controlled studies.

- A key difference between humans and laboratory animals relative to boric acid toxicity is the large zinc stores in humans. Zinc has been shown to protect against developmental and fertility effects of boric acid in animal and *in vitro* studies. The protective effect of the large zinc stores in the human body against boric acid associated toxicity explains in part the absence of effects in humans exposed to high levels of boron.

- A low intrinsic hazard of boron in humans is supported by the lack of an endocrine-related mechanism for the fertility and developmental effects in laboratory animals, the numerous studies showing the physiological importance for boron, evidence for the homeostatic control of boron in humans and mammals, and that boron meets criteria of essentiality (nutrient). A low U.S. EPA Toxicological Priority Index (ToxPi) score (a weight-of-evidence score focusing on estrogen, androgen and thyroid pathways) (Reif et al. 2010), the fact that boric acid is not mutagenic and is not carcinogenic in either mice or rats support the conclusion that boric acid is not an endocrine active substance.

Based on the total weight of evidence, the data show that it is improbable that boric acid will cause reproductive or developmental effects in humans.

Boric acid shows adverse effects on fertility as well as developmental toxicity in laboratory animals. Therefore a classification as reproductive toxicant is warranted. Nevertheless for classification in category 1 the available data must allow "*a strong presumption that the substance has the capacity to interfere with reproduction in humans*." Further it is described that "*when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate*" ((EC) No 1272/2008, Table 3.7.1(a)).

Based on mechanistic data and contradicting results from animal and human data, the definition for category 2 is the most appropriate: "Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1."

Based on a total weight of evidence, Repr. Category 2 H361d: suspected of damaging the unborn child is considered the appropriate classification. Extensive evaluations of sperm parameters in highly exposed workers have demonstrated no effects on male fertility justifying the removal of the fertility classification. While no developmental effects have been seen in highly exposed populations, epidemiological studies of developmental effects are not as robust as the fertility studies, warranting the Category 2 H361d.

Concluding the arguments presented above, classification of disodium octaborate tetrahydrate as suspected human reproductive toxicant, category 2 (H361d: Suspected of damaging the unborn child) is regarded appropriate.

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This classification accommodates for both the positive findings in laboratory animals and the absence of significant effects in humans.

Other Sections

Part B Section 8 Annex I p72: Commission Working Group of Specialised Experts in the fields of Reprotoxicity

The SE summary report noted that an expert reported of a case of boric acid poisoning of babies treated with baby powder on irritated skin under the nappies, also concern with people with eczemas. However, no reference was provided in the SE report.

Specific studies have been carried out using talc containing boric acid. Fisher et al (1955) investigated absorption of boric acid in a large number of babies (in an institution) who were treated regularly with talc containing 5% boric acid, using a spectrophotometric analytical technique. The study was carried out over twelve months and no absorption of boric acid was detected.

In the study on babies, both intact and skin with varying degrees of diaper rash was treated with talc with 5% boric acid. The babies were catheterized. Using the turmeric paper test, boric acid was detected in the urine only from babies who had diaper rash. However, the detection of boric acid was qualitative only and they claim never to have found evidence of boron in water or normal human urine by this method as a justification for using the method and not employing a more sensitive method. Since there is a background level of boron in water and urine this casts doubt on the reliability of their data. Since the turmeric paper test is so insensitive and is qualitative only this casts doubt on the validity of all the data.

In another study on babies, Johnstone et al. (1955) measured blood and urinary boron levels in 8 infants before, during and after the application of boric acid containing talc to intact skin using a carmine-red method analytical method. Mean control-period urine and blood boron levels were 1.5 and 0.4 ppm respectively and treatment-period urine and blood levels were 2.5 and 0.6 ppm. The authors indicate that their results show little or no absorption of boric acid from borated talc through either intact or skin that had sustained a second degree burn and support the use of boric acid-containing talc. Although, the differences between control period and treatment period results are not statistically significant, even if significant differences had been found, the results of the study would have been in considerable doubt. Comparison of paired urine and blood values show a number of cases where blood values are much higher than urine values which is extremely unlikely under normal circumstances and in the absence of impaired renal function, since urinary excretion is the route by which a very short biological half-life is maintained.

Vignec and Ellis (1954) published the first reliable data to show no absorption of boric acid from 5% boric acid powder in infants with supportable analytical data as well as background levels of boron in blood and urine consistent with current knowledge. Control infants (n = 15) isolated from boron-containing medications for 1 month had blood boric acid mean levels 0.1 (± 0.06) mg boric acid/100 cc (0.17 µg B/cc blood) while urine mean levels were 0.06 (± 0,07) mg boric acid/100 cc (0.12 µg B/cc urine). An average of 0.894 (range 0.404-1.342) g/kg of 5% boric acid powder applied to the diaper region of infants (age <6 months) 7-10 times per day (estimated by authors to represent an average of 2.33 g boric acid/day) for 1 month. The treated group (n = 41) was divided into those with normal skin (n = 29) and those with rash of "varying nature and intensity" (n = 12) and for the latter, blood samples were obtained 48-72 hours following onset of rash. Blood levels for test subjects without rash were mean 0.04 (± 0.04) mg boric acid/100 cc (0.07 µg B/cc) while those with rash had a mean blood boric acid level of 0.06 mg/100 cc blood (0.10 µg B/cc blood), indicating no absorption of boric acid.

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Studies by Friis –Hansen et al. 1982; Nielsen 1970; Stuttgen et al. 1982; Draize & Kelley 1959; Pfeiffer et al. 1945 show that dermal absorption of boric acid is minimal, primarily affected by the boric acid preparation. Boric acid incorporated in a water-based jelly will show an increase in dermal absorption. Skin conditions, such as erythema, eczema, or psoriasis are of minor importance to boron skin permeability, as compared to the characteristics of the vehicle (Stuttgen et al. 1982). Water-based jelly of boric acid is not sold or manufactured; therefore dermal absorption based on application of boric acid incorporated into jelly is not relevant.

p55 Part B Section 4.12.1.1 Neurotoxicity

"No neurotoxic studies were available for the biocide evaluation. CNS depression was observed in poisoning cases. Apart from the CNS effects that occur at these very high doses there are no indications that boric acid or other borates have neurotoxic properties."

Comments:

An acute neurotoxicity study of boric acid in rats was conducted in response to U.S. EPA data requirements. Boric acid was administered to male and female rats to evaluate the potential neurotoxicity of the test substance following a single oral gavage dose (followed by a 14-day observation period). The test substance was administered in a vehicle of 1% (w/v) aqueous carboxymethyl cellulose to Group 1and 2 animals at respective dose levels of 0 or 2000 mg/kg. Experimental endpoints consisted of moribundity/mortality and clinical observations, body weights and body weight gains, functional observation battery and motor activity evaluations, post-fixation brain weights, and gross necropsy and neurohistopathologic observations.

Administration of the test substance at 2000 mg/kg resulted in no mortality or adverse clinical signs. Total body weight gain in the boric acid-treated male rats was decreased 16% compared to the control group at the end of the 14-day observation period. Functional observation battery and motor activity evaluations did not show any evidence of neurotoxicity in either sex. No neurohistopathology findings (gross lesions or treatment-related microscopic findings) were observed in either sex. In conclusion, a single oral (gavage) dose of boric acid at a level of 2000 mg/kg to male and female rats was not neurotoxic. (Johnson 2011)

p56 Part B Section 4.12.1.2 Immunotoxicity

"No immunotoxic studies were available for the biocide evaluation. There are no indications from acute and repeated dose studies that borates have immunotoxic properties."

Comments:

A 28-day oral immunotoxicity study in mice of boric acid was conducted to meet U.S. EPA data requirements. Boric acid was tested in the sheep red blood cell (SRBC) hemolytic plaque assay to determine if it had the capacity to cause adverse effects on the functional responsiveness of major components of the immune system (Curry 2012). The hemolytic plaque assay, used in the current study design, determines the functionality of the T -cell dependent antibody response (TDAR). In this study SRBCs were used to induce IgM antibody-forming B-lymphocytes in vivo in rodent splenocytes. The extent of induction of splenic lymphocytes was quantitated by assessing splenocytes for their ability to lyse SRBCs in the presence of guinea pig complement.

The test substance was administered daily to four groups of 10 female B6C3F 1 mice by oral gavage, at dose levels of 250, 500, 750 and 1000 mg/kg for 28 consecutive days. A vehicle control group of 10 animals received only the

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vehicle (1% carboxymethylcellulose). Four days prior to sacrifice, all animals were given an intravenous injection (IV) of approximately 3 X 10^8 SRBCs. The positive control group of 10 animals (which also received the IV injection of approximately 3 X 108 SRBCs 4 days prior to splenocyte harvest) was given an intraperitoneal dose of cyclophosphamide (80 mg/kg bw) approximately 24 hours prior to sacrifice. Endpoints monitored in the study, in addition to hemolytic plaque formation, consisted of clinical observations, body weight, food consumption, and spleen and thymus weight.

There were no deaths and no adverse clinical observations due to test substance treatment. Body weight, body weight changes and food consumption were normal. There were no statistically significant reductions in absolute spleen or thymus weights in the boric acid-treated group relative to the vehicle control animals. There was a statistically significant reduction in plaque forming cells in the positive control (cyclophosphamide) animals compared to the vehicle control animals. There was a reduction in plaque formation in all boric acid-treated groups compared to the vehicle control animals. The reductions in plaque formation appeared to be dose-dependent, at least up to the second highest dose level. However, none of the reductions in plaque formation were statistically significant, and therefore it is concluded that boric acid was not immunotoxic under the conditions of exposure described in this report.

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