Status Report on the Systematic Review of Quaternary Ammonium Disinfectant Compounds for Developmental and Reproductive Toxicity

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Quaternary ammonium compounds (QUATs) are common ingredients in cleaning products, disinfectants, hand sanitizers, hand wipes and other personal care products. They have been used for more than half a century and are generally considered to be safe when used according to directions. Recently, a series of papers from the laboratory of Hrubec, Melin and colleagues have reported that exposure to QUATs is responsible for reduced fertility in mice and neural tube defects (NTDs) in both mice and rats. The validity of these findings conflict with the conclusions of various regulatory bodies on the risks due to QUATs exposure when these products are used appropriately.

In order to help resolve whether QUATs interfere with the reproductive and developmental processes in mammals, a Teratology Working Group with expertise in developmental and reproductive toxicology has been empaneled and tasked by the Household and Commercial Products Association (HCPA) to perform a systematic review of the available data. This review concentrates on ADBAC, DDAC and benzalkonium chloride.

The Working Group conducted a search of the published scientific literature using Medline. The search terms are provided in Appendix A. Through a review of titles and abstracts, the search results were culled to a body of potentially relevant in vivo studies conducted in mammalian species. To these studies, unpublished developmental and reproductive toxicology studies of ADBAC and DDAC were added.

This search identified 789 potential articles. Based on review of the titles and abstracts, the body of published scientific articles was reduced to 6 in vivo laboratory studies conducted in mammalian species with ADBAC, DDAC and/or benzalkonium chloride. An additional 3 papers that described in vitro/alternative model studies and/or mechanistic studies were also identified. Outside of this literature search, the dissertation by Melin was also found freely accessible via the internet, two chapters of which had been published and were identified as separate papers in the literature search. Two additional chapters reported on studies that have not been published. It was determined that these two chapters should be included in the body of literature to be assessed in the systematic review. In addition, the Working Group was granted access to the developmental and reproductive safety studies (6 definitive studies and 4 dose range-finding studies) performed in support of the registration of ADBAC and DDAC. (Access to unpublished benzalkonium chloride safety studies is forthcoming.) For the purposes of the review, the dose range-finding studies are being considered in combination with their associated definitive studies.

All studies (those published in the open literature and reports from contract research organizations [CROs]) were subjected to evaluations of quality for risk assessment purposes by

using the ToxRTool (Schneider et al, 2009), which is based on the Klimisch et al. (1997) scoring technique. The studies were evaluated independently by two authors using the ToxRTool and scored based on reported study methods and level of data documentation. Any discrepancies in scoring were discussed such that the authors came to agreement on the overall score assigned to each study. Using ToxRTool, the studies were divided into three groups: Group 1 - Reliable without restrictions; Group 2 - Reliable with restrictions; and Group 3 -Not reliable. The scores for individual papers are presented in Appendix B.

At present, scoring has identified four (4) Group 1 studies; three (3) Group 2 studies; and ten (10) Group 3 studies. Two of the Group 2 studies (reliable with restrictions) were downgraded from quality Group 1 studies based on the numbers of animals per group only. Both of these rabbit studies met contemporary guidance (1990's) at the time the studies were conducted, but the numbers of animals per group were too few according to current guidance. Summaries have been prepared or are in the process of being prepared for all 17 of the scored studies.

While the analysis of all the data is ongoing, at this point, we note that there were no test article-related developmental or reproductive endpoints in studies involving rats or rabbits among the six (6) combined unpublished (Group 1 and Group 2) studies. The one possible exception is a slight increase in dead fetuses and reduced fetal weights at the highest DDAC dose tested in the rabbit; however, the high dose values were generally without dose-response, within the expected range, and/or not statistically significant. They also occurred at a dose that was associated with significant maternal toxicity, including mortality.

Among the eight (8) Group 3 (not reliable) studies that used in vivo mammalian systems, five (5) studies form the basis for the claims of adverse reproductive effects. These five studies were conducted primarily in mice and are from the same laboratory (Hrubec, Melin and colleagues). The reported adverse effects include reduced fertility in mice, the presence of open neural tubes in mouse embryos at gestation day 10; and alterations in mouse sperm. We note the following concerning these studies:

1. Some of the methods and endpoints reported in these studies are non-standard, not reported, not well-described, and/or incorrectly calculated. For example, limited results are provided from 6-month continuous breeding periods instead of controlled single- or two-generation matings. Also, female body weights during gestation and lactation are not reported, nor some of the methods used in the experiments.
2. The studies involving "ambient" exposure did not measure or identify ADBAC or DDAC in the mice or their offspring. The authors assumed exposure because the floors of the animal rooms were washed with a disinfectant containing ADBAC and DDAC or the

rooms were reported to have been "fogged" with the disinfectant to control parasitic infestation. Animal exposure was not confirmed.

3. In the dietary (and drinking water) studies, the dietary (or drinking water) concentrations administered were not reported, mice were not weighed, and the method for determining the daily intake of nutrient gel (or water) was not described. Further, the numbers of mice per cage were not reported, although group housing was noted in some cases. For these reasons, the doses reported are not reliable.
4. The authors report open cranial neural tubes at GD 10 as "neural tube defects." We note that a neural tube defect is a condition observed at term that entails incomplete closure of the skull and/or vertebrae (which are not present on GD 10) with resultant uncovering of meninges (also not present at GD 10) and sometimes the brain or spinal cord. However, when the authors allowed litters to go to term, neural tube defects were not seen in any of their studies. This suggests that either the GD 10 observations were due to developmental delays (which were corrected by the end of gestation) or the embryos may have been dying (which would result in resorptions, but not malformations). Regardless, there were no neural tube defects in term-fetuses.

Our complete assessment will include reviews of two additional OECD 414 studies and two additional OECD 416 studies conducted on QUATs. These studies have been reviewed and accepted by ECHA and the Biocidal Products Committee supporting European biocide registration and uses. Additional analysis is underway to determine if these data can support claims of adverse effects on reproduction that were not detected in the robust reproductive and safety studies conducted on QUATs that have already been reviewed by the U.S. EPA and regulatory agencies worldwide. Finally, although review and summarization of the individual studies we have on hand is almost complete and some of the issues with the different studies have been identified as outlined above, written documentation of our full assessment has yet to be prepared. This assessment will ultimately be submitted for peer-review and publication.

Text References

Klimisch, H. J., Andreae, M. and Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Reg. Toxicol. Pharm. 25:1–5.

Schneider, K., Schwarz, M., Burkholder, I., Kopp-Schneider, A., Edler, L., Kinsner-Ovaskainen, A., Hartung, T. and Hoffmann, S. (2009). "ToxRTool", a new tool to assess the reliability of toxicological data. Toxicol. Lett. 189:138–144.

Appendix A

Search terms for Literature Search

The following search terms were used to conduct a search of published literature:

"Quaternary ammonium compounds" OR Quats OR "Didecyl dimethyl ammonium chloride" OR DDAC OR "Alkyl dimethyl benzyl ammonium chloride" OR ADBAC OR "Benzalkonium chloride"

OR

(RN(7173-51-5 OR 61789-71-7 OR 8001-54-5 OR 68424-85-1))

AND

Gestation OR Pregnancy OR Prenatal OR Fetal OR Embryonic OR Maternal OR "Developmental toxicity" OR Reproduction OR "Neural tube defect"

A system algorithm was used to remove as many duplicate citations as possible.

Appendix B

Quality Scores for Studies

	Paper	Reference	Quality score
1	Unpublished – ADBAC two-generation study	Neeper-Bradley, 1990	1
2	Unpublished ADBAC rabbit developmental toxicity study	Neeper-Bradley and Kubena, 1992	2
3	Unpublished ADBAC rat developmental toxicity study	Neeper-Bradley, 1992	1
4	Unpublished DDAC two-generation study	Neeper-Bradley, 1991	1
5	Unpublished DDAC rabbit developmental toxicity study	Tyl, 1989	2
6	Unpublished DDAC rat developmental toxicity study	Neeper-Bradley, 1991	1
7	Published – Embryotoxicity of benzalkonium chloride in vaginally treated rats	Buttar, 1985	3
8	Published (mechanistic) – Multiomics investigation reveals benzalkonium chloride disinfectants alter sterol and lipid homeostasis in the mouse neonatal brain	Herron et al., 2019	3
9	Published – Ambient and dosed exposure to quaternary ammonium disinfectants causes neural tube defects in rodents	Hrubec et al., 2017	3
10	Published – Teratogenicity study of dicetyldimethylammonium chloride in mice	Inoue, 1980	3
11	Published – Exposure to common quaternary ammonium disinfectants decreases fertility in mice	Melin et al., 2014	3
12	Published – Quaternary ammonium disinfectants cause subfertility in mice by targeting both male and female reproductive processes	Melin et al., 2016	3
13	Published – Disinfectant compounds ADBAC+DDAC exhibit concentration and temporally dependent reproductive toxicity in-vitro and in-vivo	Melin, 2015 dissertation – Chapter 4	3
14	Published – Direct and in-utero exposure to quaternary ammonium disinfectants alters sperm parameters and RNA expression of epigenetic enzymes in the testis of male mice	Melin, 2015 dissertation – Chapter 5	3
15	Published – Effects of benzalkonium chloride on pregnant mice	Momma et al., 1987	3
16	Published – Effect of hatching egg sanitizers on embryonic survival and hatchability of turkey eggs from different lines and on egg shell bacterial populations	Sacco et al., 1989	3
17	Published (mechanistic) – Benzalkonium chloride, benzethonium chloride, and chlorxylenol - three replacement antimicrobials are more toxic than triclosan and triclocarban in two model organisms	Sreevidya et al., 2018	2