



Pamela G. Bailey  
President & CEO

May 31, 2005

Dr. Scott A. Masten  
Office of Chemical Nomination and Selection  
NIEHS/NTP  
111 T.W. Alexander Drive  
P.O. Box 12233  
Research Triangle Park, NC 27709

RE: Request for Additional Information on Toxicological Study Nominations to the National Toxicology Program (70 Federal Register 23877): Butylparaben

Dear Dr. Masten,

The Cosmetic, Toiletry, and Fragrance Association<sup>1</sup> (CTFA) appreciates the opportunity to provide additional information on the above referenced topic. Butylparaben is used as a preservative within the personal care products industry, and thus its nomination for study is of interest to CTFA members. CTFA has recently conducted studies on butylparaben to evaluate dermal absorption and metabolism, and male reproductive toxicity. These studies are being submitted to the National Toxicology Program in response to the request for additional information on nominated substances.

The skin penetration/metabolism study measured dermal penetration of butylparaben (and also methylparaben) in viable human and rat skin in vitro. A cosmetic vehicle (oil-in-water emulsion) was used, and receptor fluid was analyzed for metabolites. Three human and three rat donors were included, and the human skin was dermatomed. In addition, a pilot study was conducted using one human donor and full thickness skin to see what effect including the entire dermis would have on metabolism. The study results demonstrate that while penetration is significant, the skin has substantial capability to metabolize parabens, therefore limiting systemic exposure. In the full thickness pilot study, metabolism of butylparaben to the major metabolite p-hydroxybenzoic acid was seen to be essentially complete. Additional work which will include full thickness skin from two more human donors is in progress.

---

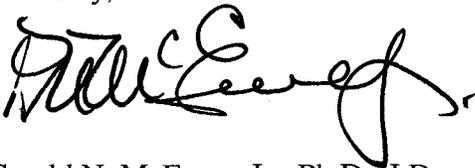
<sup>1</sup>Based in Washington, D C , CTFA is the trade association representing the cosmetic, toiletry, and fragrance industry in the United States and globally. Founded in 1894, CTFA has a membership of nearly 600 companies including manufacturers, distributors, and suppliers for the vast majority of finished personal care products marketed in the United States

The male reproduction study was undertaken following reports suggesting butylparaben had the potential to affect the development of the male reproduction system and decrease sperm production in rodents.<sup>2</sup> A study was conducted under GLP conditions using the same experimental design as the published study in rats, but with a greater number of animals and additional endpoints including detailed histopathological examination of reproductive tissues, and more extensive hormonal analyses. Because the major route of exposure for butylparaben from personal care products is dermal, consideration was given to dosing via the dermal route. However, due to solubility issues with butylparaben which would limit the dose that could be tested, the oral route was chosen for the study.

No effects were seen at doses up to 1000 mg/kg/day (the highest dose tested). A table providing a side-to-side comparison of the protocol and results of this study compared to the Oishi study is included in this data submission.

Thank you for the opportunity to provide information relevant to the butylparaben study nomination. The reports for the penetration/metabolism studies, and the reproduction study, along with the comparison document referred to above, are enclosed.

Sincerely,



Gerald N. McEwen, Jr., Ph.D., J.D.  
Vice President - Science



---

<sup>2</sup> Oishi, S. (2001) Effects of butylparaben on the male reproductive system in rats. Toxicol Ind Health, **17**: 31-39;  
Oishi, S. (2002) Effects of butyl paraben on the male reproductive system in mice. Arch Toxicol, **76**: 423-429.

### Comparison of Oishi (2001) Butylparaben Study With the Charles River-Argus (2005) Butylparaben Study

Endpoint	Oishi (2001) Study	Charles River - Argus (2005) Study
PROTOCOL		
Rat strain/source	male Crj:Wistar/Charles River, Japan	male Crl:Wistar/Charles River, North Carolina
Housing	individually housed in wire-bottom stainless steel cages, 21-25°C, RH 55 ± 5%, 12-hour light-dark cycle	individually housed in wire-bottom stainless steel cages, 19-23°C, RH 70-74%, 12-hour light-dark cycle
Diet	CE-2 feed, Clea, Tokyo Japan isoflavone levels reported in mouse study Oishi (2002) - which were originally reported in a bisphenol A paper	CE-2 feed, Clea, Tokyo Japan diet analyzed for isoflavones and p-aminobenzoic acid
Age/Weight at start of treatment	19-21 days/49.9 ± 3.01 g	21 days/28.3-48.7 g
Number of male rats per treatment group	8	16
Dietary concentrations of Butylparaben	0, 0.01%, 0.1%, 1.0% (0, 100, 1000, 10000 ppm)	0, 100, 1000, 10000 ppm
Duration of treatment	8 weeks	56 days (8 weeks)
In-life measurements	body weight (daily) food consumption (daily)	body weight (daily) food consumption (2x/week) week 3, 5, 7 orbital sinus blood draw for measurement of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) blood samples were collected at same time of day each week



Necropsy	organ weights: testes, epididymides, ventral prostates, preputial glands, seminal vesicles with coagulation gland	organ weights: liver, adrenal glands (paired), thyroid, pituitary, right testis, left testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid), prostate (ventral and dorsal)
Sperm counts/Daily sperm production (DSP)	right testis	left testis
Sperm motility/concentration/ morphology	motility/morphology not determined. Concentration of sperm in cauda epididymides determined	computer-assisted sperm analysis (CASA) used; motility assessed from sample from the left vas deferens; concentration and morphology of sperm from left cauda epididymis
Hormone analysis	testosterone (kit from Oxford Biomedical Research)	ELISA methodology used to measure all hormones testosterone (kit Biomedica), LH (kit Amersham Pharmacia Biotech), FSH (Amersham Pharmacia Biotech kit)
Histopathology reproductive organs	not completed	remaining left epididymis, right epididymis, right testis, prostate, seminal vesicles control and high concentration groups, testicular staging was also completed
RESULTS		
Body weights at study termination	no significant differences 0                    396 ± 34.5 100                  393 ± 21.5 1000                400 ± 19.9 10000              378 ± 19.0	no significant differences 0                    300.8 ± 32.0 100                294.1 ± 31.6 1000              312.5 ± 33.5 10000            301.2 ± 31.4

Mortality/early sacrifice	no premature deaths reported	2 rats (1 at 0 ppm, 1 at 100 ppm) were sacrificed because of eye lesions from retro-orbital bleeding
Consumed doses (means)	10.4, 103, 1026 mg/kg/day	10.9, 109.3, 1087.6 mg/kg/day
Isoflavone levels	in µg/g diet daidzin 125, glycirin 26.7, genistin 146, daidzein 3.95, glycitein 25.4, genistein 3.87 (total = 330.92)	in 2 lots of diet 912, and 924 µg/g diet; p-aminobenzoic acid 6.92 and 4.37 ppm
Reproductive organ weights	no effects on testes, ventral prostates, preputial glands absolute weights of the epididymis and seminal vesicles with coagulation glands were significantly lower at 10000 ppm relative weights of epididymis decreased in dose-dependent manner - significant at 1000 ppm at above Relative epididymides weights 0                    0.267 ± 0.0191 100                0.247 ± 0.0162 1000              0.237 ± 0.134 10000            0.233 ± 0.0127	no significant effects weights of left epididymis, cauda epididymis and right epididymis measured separately
DSP	significantly (p<0.05) decreased at all doses 0                    40.0 ± 5.86 100                33.3 ± 4.9 1000              28.3 ± 2.71 10000            24.5 ± 5.43	no effects 0                    36.82 ± 11.38 100                31.81 ± 13.8 1000              31.09 ± 12.53 10000            32.83 ± 15.37

Sperm counts in the cauda epididymis	<p>significantly decreased at all doses</p> <table> <tr> <td>0</td> <td>56.0 ± 12.9</td> </tr> <tr> <td>100</td> <td>41.4 ± 8.22</td> </tr> <tr> <td>1000</td> <td>41.6 ± 9.33</td> </tr> <tr> <td>10000</td> <td>32.6 ± 9.80</td> </tr> </table>	0	56.0 ± 12.9	100	41.4 ± 8.22	1000	41.6 ± 9.33	10000	32.6 ± 9.80	<p>no effects</p> <table> <tr> <td>0</td> <td>49.7 ± 25.0</td> </tr> <tr> <td>100</td> <td>78.3 ± 75.8</td> </tr> <tr> <td>1000</td> <td>56.4 ± 28.1</td> </tr> <tr> <td>10000</td> <td>49.4 ± 16.8</td> </tr> </table>	0	49.7 ± 25.0	100	78.3 ± 75.8	1000	56.4 ± 28.1	10000	49.4 ± 16.8
0	56.0 ± 12.9																	
100	41.4 ± 8.22																	
1000	41.6 ± 9.33																	
10000	32.6 ± 9.80																	
0	49.7 ± 25.0																	
100	78.3 ± 75.8																	
1000	56.4 ± 28.1																	
10000	49.4 ± 16.8																	
Sperm morphology/motility	not measured	no effects																
Hormone analysis	<p>testosterone dose-dependent decrease that was statistically significant at the 2 highest dose</p> <p>Values (ng/ml) estimated from Figure 2</p> <table> <tr> <td>0</td> <td>9</td> </tr> <tr> <td>100</td> <td>7.5</td> </tr> <tr> <td>1000</td> <td>6</td> </tr> <tr> <td>10000</td> <td>3</td> </tr> </table> <p>(control values from Oishi's propylparaben study 9.08 ± 2.12 ng/ml; control values from Oishi's methyl-, ethylparaben study 11.9 ± 2.08 ng/ml)</p>	0	9	100	7.5	1000	6	10000	3	<p>no biologically important differences that could be related to treatment were observed; some statistically significant observations were noted - reductions in testosterone at 1000 and 10000 ppm after 3 weeks of exposure, significant increase in testosterone at 10000 ppm after 9 weeks of exposure; reductions in LH at 5 weeks of exposure at 100 and 10000 ppm which were not dose-related</p> <p>Values (ng/ml) at week 9</p> <table> <tr> <td>0</td> <td>1.085 ± 0.430</td> </tr> <tr> <td>100</td> <td>1.485 ± 0.767</td> </tr> <tr> <td>1000</td> <td>1.031 ± 0.575</td> </tr> <tr> <td>10000</td> <td>1.86 ± 0.968 (p&lt;0.01)</td> </tr> </table>	0	1.085 ± 0.430	100	1.485 ± 0.767	1000	1.031 ± 0.575	10000	1.86 ± 0.968 (p<0.01)
0	9																	
100	7.5																	
1000	6																	
10000	3																	
0	1.085 ± 0.430																	
100	1.485 ± 0.767																	
1000	1.031 ± 0.575																	
10000	1.86 ± 0.968 (p<0.01)																	
Histopathology	not completed	no adverse findings in reproductive organs																