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within***

 **FERRO.**

**Ferro Corporation
Comments regarding BBP
before the
OEHHA Cancer Identification Committee**

**Sacramento, CA
December 5, 2013**

**Alan Olson
Ferro Corporation
Corporate Product Stewardship**

Ferro Corporation

- ✓ Headquartered in Ohio.
- Monsanto began making Butyl Benzyl Phthalate (BBP) in the 1960's; Solutia sold business to Ferro.
- Ferro produces BBP at sites in New Jersey and Antwerp, Belgium.
- Ferro sells BBP for industrial use as an additive for several types of plastics.
- Applications are largely building and construction materials.
 - No BBP in toys and child care articles.
 - Food contact uses are not promoted.
- Ferro does NOT sell BBP to consumers.
- BBP listed under Proposition 65 in 2005 for developmental toxicity.

Butyl Benzyl Phthalate

- ✓ **All available relevant evidence to be considered in a “Weight of Evidence” approach.** (Guidance Criteria For Identifying Chemicals For Listing As “Known To The State To Cause Cancer”, March 2001)
- ✓ We recognize the CIC must make its own evaluation, but would note:
 - IARC considered the same animal bioassays in 1999 as now under review, and did not classify BBP for cancer (“not classifiable”, Group 3).
 - BBP not classified for cancer by the European Chemicals Bureau in its risk assessment of BBP. (“...BBP can be considered as a non-genotoxic substance.” “Overall, BBP is considered as a non-carcinogenic substance.” 2008)
 - NTP, which conducted the three BBP bioassays (1982, 1997a, 1997b), has not considered BBP for listing in its Report on Carcinogens.

Butyl Benzyl Phthalate

- Ferro Corp. has submitted a comprehensive review of the mutagenicity and carcinogenicity data for BBP to the CIC.
- Information compiled by
 - John Butala, DABT, who has conducted toxicology studies on phthalates, including BBP, for more than twenty years.
 - Errol Zeiger (director of genetic toxicology testing at FDA and NTP)
 - Eugene McConnell (toxicology research and testing at NTP)
- **The Weight of Evidence is that BBP should not be listed as “known to the state to cause cancer”.**



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Criteria for Evaluation of BBP by the Cancer Identification Committee

**Sacramento, CA
December 5, 2013**

**Ann Claassen
Latham & Watkins LLP
Counsel to Ferro Corporation**

Proposition 65 Statutory Definition

- “A chemical is known to the state to cause cancer ...
- “within the meaning of this chapter
- “if in the opinion of the state's qualified experts
- “it has been clearly shown
- “through scientifically valid testing
- “according to generally accepted principles
- “to cause cancer”

--California Health & Safety Code section 25249.8(b)

Guidance Criteria for Identifying Chemicals for Listing as “Known to the State to Cause Cancer”

- http://www.oehha.ca.gov/prop65/policy_procedure/pdf_zip/revcriteria.pdf
- “The criteria included herein shall be utilized by the ... CIC”
- “In evaluating the sufficiency of available data, a “weight-of evidence” approach shall be used to evaluate the body of information available for any given chemical.”
- “The body of evidence shall include all evidence bearing on the issue of carcinogenicity shown through scientifically valid testing according to generally accepted principles.”
- “CIC members may make judgements utilizing other, more indirect, scientifically valid observations ... although changes induced in whole mammals must be considered more pertinent.”

[emphasis added]

Guidance Criteria, cont.

- In general, animal evidence should show excess tumors “in the two genders of a species, in two distinct species, or in two different experiments carried out in two different laboratories under different protocols”
- Exceptions may allow lesser evidence to suffice:
 - “if the malignant tumors occurred to an unusual degree with respect to frequency, type, location, age at onset, or low dosage, or in a strain not otherwise prone to such tumors”
 - “if heavily supported by the indirect evidences”
- Increases in tumors should be such that “[t]he observed difference cannot be reasonably explained by chance”

[emphasis added]



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**Butyl Benzyl Phthalate
Ferro Corporation Comments
for Consideration by the OEHHA
Carcinogen Identification Committee**

**Sacramento, CA
December 5, 2013**

**J.H. Butala, DABT
Consultant to
Ferro Corporation**

Overview

- **The weight of evidence is not sufficient for a determination that BBP has been clearly demonstrated to cause cancer.**
- Three NTP reports – each tumor type observed in only one sex of one species; tumor findings not replicated across the studies.
- The weight of evidence is that BBP is not genotoxic.
- Two limited human epidemiology studies – no association of BBP with breast cancer incidence.
- Ferro comments supported by the opinions of Dr. Errol Zeiger and Dr. Eugene McConnell.

Rodent Carcinogenicity Testing

- Report of Eugene McConnell, DVM, MS, DACVP, DABT
 - 40 year career as a pathologist
 - Former head of the Pathology Branch at the National Toxicology Program (NTP)
 - Directed the NTP Toxicology Research and Testing Program
- Dr. McConnell reviewed the OEHHA HID and the three NTP reports on BBP (1982, 1997a and 1997b).
- Conclusion: The weight of evidence does not support listing BBP as a carcinogen under Proposition 65.
- Summary of points from Dr. McConnell's report follow.

MNCL in Female Rats

- Although there was a statistically significant increase in MNCL in female rats in NTP (1982), there was no such increase in female rats in NTP (1997a) or NTP (1997b).
- Increased MNCL was not seen in male rats in any of the studies, nor in either sex in the study of mice (NTP, 1982).
- Further, MNCL is a lesion of questionable significance for human health assessment.

MONONUCLEAR CELL LEUKEMIA				
STUDY	TEST SPECIES	MALE FINDINGS	FEMALE FINDINGS	COMMENT
NTP – 213	RAT	INADEQUATE FOR CLASSIFICATION	POSITIVE – OEHA NEGATIVE NTP	HIGH DOSE ONLY
NTP – 458	RAT	NEGATIVE	NEGATIVE	
NTP – 460	RAT	NEGATIVE	NEGATIVE	RESTRICTED FEED - 2 YEARS
NTP – 460	RAT	NEGATIVE	NEGATIVE	RESTRICTED FEED- LIFETIME
NTP – 213	MOUSE	NEGATIVE	NEGATIVE	

Pancreatic Acinar Cell Tumors

- Although there was a statistically significant increase of pancreatic acinar cell tumors in male rats in NTP (1997a), there was no increase in male rats in NTP (1997b).
- There was no increase in female rats in any of the studies (NTP, 1982; 1997a; 1997b).
- There was no observation pancreatic acinar cell tumors in mice of either sex (NTP, 1982).

ACINAR PANCREAS TUMORS				
STUDY	TEST SPECIES	MALE FINDINGS	FEMALE FINDINGS	COMMENT
NTP – 213	RAT	INADEQUATE FOR CLASSIFICATION	NEGATIVE	
NTP – 458	RAT	POSITIVE	NEGATIVE	HIGH DOSE ONLY
NTP – 460	RAT	NEGATIVE	NEGATIVE	RESTRICTED FEED - 2 YEARS
NTP – 460	RAT	NEGATIVE	NEGATIVE	RESTRICTED FEED- LIFETIME
NTP – 213	MOUSE	NEGATIVE	NEGATIVE	

Adrenal Medulla Tumors

- HID states there was an increase in adrenal medulla pheochromocytoma in female rats in NTP (1982), but this was not the finding of NTP itself.
- There was no increase in these tumors observed in male or female rats in the 1997 studies (NTP, 1997a; 1997b).
- There was no increase in adrenal medulla pheochromocytoma in mice of either sex (NTP, 1982).

ADRENAL MEDULLA PHEOCROMOCYTOMA				
STUDY	TEST SPECIES	MALE FINDINGS	FEMALE FINDINGS	COMMENT
NTP – 213	RAT	INADEQUATE FOR CLASSIFICATION	POSITIVE-OEHHA NEGATIVE -NTP	HIGH DOSE ONLY
NTP – 458	RAT	NEGATIVE	NEGATIVE	
NTP – 460	RAT	NEGATIVE	NEGATIVE	RESTRICTED FEED - 2 YEARS
NTP – 460	RAT	NEGATIVE	NEGATIVE	RESTRICTED FEED- LIFETIME
NTP - 213	MOUSE	NEGATIVE	NEGATIVE	

Urinary Bladder Tumors

- Incidence of urinary bladder transitional cell tumors in female rats in NTP (1997a) considered “equivocal evidence” of carcinogenicity. NTP did not perform statistical analysis on this “uncertain effect”.
- Increase in urinary bladder transitional cell tumors in female rats in NTP (1997b) was not statistically significant, and the NTP did not consider this a positive finding.
- McConnell considered the bladder changes likely due to chronic irritation (32 months) via physical damage to the epithelium.
- No increases were observed for this tumor in female rats in the other studies, in male rats in any study, nor in male or female mice.

URINARY BLADDER – TRANSITIONAL CELL TUMOR				
STUDY	TEST SPECIES	MALE FINDINGS	FEMALE FINDINGS	COMMENT
NTP – 213	RAT	INADEQUATE FOR CLASSIFICATION	NEGATIVE	
NTP – 458	RAT	POSITIVE	EQUIVOCAL EVIDENCE -NTP	24,000 PPM ONLY STATS NOT REORTED
NTP – 460	RAT	NEGATIVE	NEGATIVE	RESTICTED FEED 2 YEARS
NTP – 460	RAT	NEGATIVE	NEGATIVE - NTP POSITIVE – OEHHA P>0/5	RESTRICTED FEED- LIFETIME 24,000 PPM
NTP – 213	MOUSE	NEGATIVE	NEGATIVE	

Genetic Toxicology

Study Type	Number of Positive Results	Number of Negative Results
In vitro mutation or DNA damage in prokaryotic cells	0	10
In vitro mutation, DNA damage in mammalian cells	3	6
In vitro chromosome damage in mammalian cells	0	2
DNA damage in vivo (rodents)	1	0
Chromosome damage in vivo (rodents)	2	5
Non-mammalian gene mutation	0	2
Mammalian cell transformation	1	2
TOTAL	7	27

Genetic Toxicology, cont.

- Report of Errol Zeiger, Ph.D.
 - 31-year career in genetic toxicology
 - Directed genetic toxicology testing at FDA, then at NTP
 - Former Editor-in-Chief of Environmental and Molecular Mutagenesis
- Neither BBP or its monophthalate metabolites possess structural alerts for DNA reactivity.
- Tests that reported positive results are weakened by:
 - Inappropriate route and systemically toxic doses.
 - Lack of availability of testing details, e.g., protocol, full results and data tables.
 - Questionable level of confidence and reproducibility in positive tests.
- Predominance of fully-reported negative results for each genotox endpoint.
- BBP should not be considered genotoxic.

Human Cancer Studies

- Two breast cancer epidemiology studies are available which looked at an association between BBP-exposure and an increased incidence of breast cancer (Aschengrau, et al., 1998; Lopez-Carillo, et al., 2010).
- Both studies were negative for an association of breast cancer and BBP exposure.
- Both studies had an adequately large population to allow for statistical significance in data analysis. Subjects in both studies had documented exposure to BBP.
- One of the studies (Lopez-Carillo, et al., 2010) showed a negative association between breast cancer risk and exposure to BBP.

Weight-of-Evidence Conclusion

- There is no consistency in tumor findings with regard to occurrence in animal species or sexes.
- Observations of BBP-induced tumors in rodents were not replicated.
- WOE is that BBP is not genotoxic.
- Two independent, published reports of BBP carcinogenicity studies in humans found no positive association of BBP with breast cancer; one reported a negative association.
- The Weight-of-Evidence does not support listing BBP as a carcinogen under Proposition 65.

Supplemental slides

Mode-of-Action

- The HID proposes several carcinogenicity Modes-of-Action for BBP, including activation of cellular receptors (PPAR, ER, AR, AhR) and downstream effects on gene and protein expression.
- The tumor types that could be anticipated from carcinogenic activity via these MoA's have not been observed in any apical animal bioassays of BBP nor have they been seen in the epidemiology data for BBP.
- Specifically, no mammary tumors, no uterine or ovarian tumors, no testicular tumors have been associated with BBP in any mammalian bioassay.

Genotoxicity “positives”

1. Single strand DNA breaks in HepG2 cells in vitro
 2. SCE and chromosome aberration in mice in vivo
 3. Apurinic sites in mouse osteoblasts in vitro
 4. DNA-protein crosslinks in rat hepatocytes in vitro
 5. DNA-protein crosslinks in mouse liver cells in vivo
- Views of Dr. Zeiger on these observations follow.

1. Single strand DNA breaks in HepG2 cells in vitro

- Comet Assay sensitive to cells undergoing apoptosis or necrotic cell death.
- DNA degradation is part of cell death.
- Authors reported apoptosis concomitant with DNA strand breaks in their assay.
- DNA strand breaks can be the result of damage by a reactive chemical species

OR

an effect of a non-DNA directed apoptotic response.

2. SCE and chromosome aberration in mice in vivo

- Both endpoints were induced following ip injection.
- ip injection is not a relevant route of exposure to BBP.
- The positive dose was 5000 mg/kg which is almost double the ip LD50 in mice for BBP, suggesting a secondary effect of toxicity.
- Because SCE can be induced by direct damage to the chromosome or by interference with mitosis it is no longer recommended as a test for DNA interaction.

3. Apurinic sites in mouse osteoblasts in vitro

- Positive in mouse but not rat.
- Author's conclusion was based on indirect evidence (enzyme induction) associated with cytotoxicity.
- The association of DNA strand breaks with apoptosis was not addressed.
- The report of positive effect was limited to a conference abstract with no details on methodology and no data presented.

4&5. DNA-protein crosslinks in rat hepatocytes in vitro and in mouse liver cells in vivo

- DNA-protein crosslink (in vitro and in vivo) were reported in a conference abstract – no data or description of methodology presented.
- No chemical analysis of the crosslink to differentiate between association with BBP or an oxygen species was reported.