UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

[redacted]

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MEMORANDUM

SUBJECT: Mutagenicity Hazard Review of P08-88

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1/18/08

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I. SUMMARY

P08-88 is not a gene mutagen in two species of bacteria either without or with activation. It is a chromosome mutagen in mammalian cells in culture both without and without activation.

Based upon the data on the PMN itself and also upon analogues, P08-88 is or may be: (a) not a gene mutagen in two species of prokaryotes and in mammalian cells in culture; (b) a chromosome mutagen in mammalian cells in culture, and; (c) an inducer of DNA effects in human cells *in vivo*. Additional mutagenicity testing for the P08-88 is advisable: a mammalian *in vivo* micronucleus test (guideline OPPTS 870.5395/OECD 474) by the intraperitoneal route of

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exposure. If the *in vivo* micronucleus study is positive, additional mutagenicity and/or carcinogenicity testing may be indicated.

II. STRUCTURES OF PMN 08-88 AND ANALOGUES

[redacted]

P08-88

[redacted]

III. BASES FOR THE CONCLUSIONS

PMN 08-88 is [redacted]. There were mutagenicity data submitted with the PMN on bacterial reverse mutation and *in vitro* chromosome aberrations:

P08-88 was tested in the bacterial reverse mutation assay, as reported in "Reverse Mutation Test using Microorganisms", conducted by [redacted], [redacted] study dated June 28, 2005. The PMN, identified as [redacted], [redacted] [redacted]) was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and in *Escherichia coli* strain WP2*uvrA*, both without and with metabolic activation using phenobarbital- and 5,6-benzoflavone-induced Sprague-Dawley rat liver S9. It was tested at six dose levels ranging from 156 to 5,000 µg/plate . Dose selection was based upon a preliminary rangefinding study which detected cytotoxicity at 5,000 µg/plate, and was acceptable. The first test was confirmed in a second test. Both tests were conducted done in triplicate plates at dose levels of 156, 313, 625, 1,250, 2,500 and 5,000 µg/plate. In both tests, cytotoxicity was detected at the highest (5,000 µg/plate) dose but not at the next highest dose (2,500 µg/plate). No significant increases in reversion frequencies were observed in any test condition. Concurrent negative (distilled water) and positive controls produced appropriate responses.

The *in vitro* mammalian chromosome aberration assay, entitled "Chromosomal Aberration Test of EEA-NH4 using Cultured Mammalian Cells", was conducted by [redacted], study dated February 2006. The PMN, with identity and purity as above, was tested in Chinese hamster lung (CHL) fibroblasts both without and with metabolic activation as above. A preliminary rangefinding ("cell growth inhibition") test was conducted at nine dose levels from 14.2 to 3630 µg/plate, and the lowest dose levels at which 50% or greater growth inhibition occurred were 1400 and 1100 µg/plate for the nonactivated and activated conditions, respectively. For the mutagenicity test proper, cultures were exposed to the chemical for 4 hours both without and with activation, and harvested at 24h from initiation of exposure. Cultures were exposed to the test article at the following dose levels: 723, 868, 1040, 1250, 1500 and 1800 µg/plate without activation, and 603, 723, 868, 1040, 1250, 1500 and 1800 µg/plate with activation. Mitotic evaluations were ultimately conducted at three dose levels representing the highest dose level to demonstrate 50% cytotoxicity as well as the two dose levels immediately below it: i.e., at 1250, 1500 and 1800 µg/plate for the nonactivated assay, and 868, 1040 and 1200 µg/plate for the activated assay. Dose selection, as based upon the rangefinding study plus the cytotoxicity observed during the mutagenicity proper, was adequate. The chemical was tested in duplicate cultures. Significant increases in structural aberrations (aberration frequency increased by 10 percentage points above concurrent negative controls), along with dose responses, were detected both without and with activation. Even though significant increases occurred only at cytotoxic dose levels,

nevertheless these increases are likely of biological significance since dose responses were observed in the lower dose levels. No significant increases in numerical aberrations (polyploidy) were detected under either condition. Concurrent negative (distilled water) and positive controls (mitomycin C and cyclophosphamide for non-activated and activated conditions respectively) produced appropriate responses.

Under the conditions tested, PMN 08-88 is not gene mutagen in two species of bacteria, but it is a chromosome mutagen in mammalian cells in culture, both without and with metabolic activation.

There also are mutagenicity data on four Section 5 analogues of the PMN (all structures are in Section II):

[redacted]

All four analogues are not gene mutagens in *Salmonella* strains TA98, TA100, TA1535 and TA1537 and in *E. coli* strain WP2*uvrA* without and with metabolic activation.

Another analogue for which there are mutagenicity data is [redacted] is not a gene mutagen in mammalian V79 cells in culture (NLM 2007), but it induces DNA effects in the form of sister chromatid exchanges (SCEs) in human lymphocytes *in vivo* (Tucker et al 1993).

These data are summarized in the table on the following page.

MUTAGENICITY OF P08-88 AND ANALOGUES

effect \ chemical	[redacted]					
gene mutations						
Salmonella	neg wo & w	neg wo & w	neg wo & w	neg wo & w	neg wo & w	
Escherichia coli	neg wo & w	neg wo & w	neg wo & w	neg wo & w	neg wo & w	
V79 *in vitro*						neg
chromosome mutations						
chromosome aberrations *in vitro*	pos wo & w w dr CHL					
DNA effects						
SCE *in vivo*						HL pos

Abbreviations used in Table:

CHL = Chinese hamster lung fibroblasts; dr = dose response; HL = human lymphocytes; neg = negative; pos = positive; SCE = sister chromatid exchange; V79 = Chinese hamster V79 cells, w = with activation; wo = without activation

Thus, based on data on the PMN itself and upon analogues, P08-88 is or may be: (a) not a gene mutagen in two species of prokaryotes and in mammalian cells in culture; (b) a chromosome mutagen in mammalian cells in culture, and; (c) an inducer of DNA effects in human cells *in vivo*.

Based upon these data, additional mutagenicity testing for the P08-88 is advisable. A mammalian *in vivo* micronucleus test (guideline OPPTS 870.5395/OECD 474) by intraperitoneal injection is recommended. If this *in vivo* micronucleus study is positive, additional mutagenicity and/or carcinogenicity testing may be indicated.

IV. REFERENCES

NLM. 2007. National Institutes of Health, National Library of Medicine. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances - GENE-TOX Agent Registry. Online computer database - TOXNET file. US Dept. Health and Human Services. Bethesda MD 20894. (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX).

Tucker JD, Auletta A, Cimino MC, Dearfield KL, Jacobson-Kram D, Tice RR, Carrano AV. 1993. Sister-chromatid exchange: second report of the Gene-Tox program. Mutat. Res. 297: 101-180.