US EPA ARCHIVE DOCUMENT

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

JUL 23 1992

MEMORANDUM

SUBJECT: Phosphine - Mutagenicity Requirements

FROM: Kerry L. Dearfield, Ph.D.
Geneticist/Acting Section Head
Peer Review Section
Science Analysis Branch
Health Effects Division (H7509C)

TO: Joanne Edwards
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: William Burnam, Chief
Science Analysis Branch
Health Effects Division (H7509C)

Aluminum phosphide	[20859-73-8]	EPA Registry Code # 066501
Magnesium phosphide	[12057-74-8]	EPA Registry Code # 066504
Phosphine	[7803-51-2]	

Background

A revised registration standard for aluminum and magnesium phosphide was issued in October 1986 and the requirements in this revised standard superseded any earlier standard requirements. In this 1986 standard, mutagenicity tests were required; specifically, one test each in the categories of i) gene mutation, ii) structural chromosomal aberrations, and iii) other genotoxic effects was required. After several iterations of protocol reviews and time extensions, two tests were finally submitted to the OPP in which the test material was phosphine gas: a Salmonella assay (MRID #41434301) and an in vitro chromosomal aberrations assay with Chinese hamster ovary (CHO) cells (MRID #41434302). These two tests address the first two categories of mutagenicity testing listed above. There is still a data gap for the other genotoxic effects category and this remains a data requirement. Other available studies also have been examined and based on these and the submitted studies, additional work addressing a heritable mutagenicity concern is also required.

Printed on Recycled Paper

Submitted Studies

Two studies have been submitted to the OPP in response to the 1986 Registration Standard for aluminum and magnesium phosphide. A Salmonella assay was performed with phosphine gas and strains exposed for 48 hours. Though there were random, non-dose related, non-reproducible increases across several strains over six different experiments, overall it appears that there were not compound related increases in mutation frequency at adequately tested concentrations. This study was reviewed acceptable (DER has been generated, but no Document No. has been assigned - it does not appear to have final sign-offs yet).

The second study is an in vitro chromosomal aberrations assay with CHO cells. CHO cells were exposed to phosphine gas for 5 hours, washed, and cultures incubated for three additional incubation periods of 8, 18 or 26 hours before harvest. There was no apparent cytotoxicity as no effect on cell cycle was discernable. Small, but statistically significant increases in aberrations were found at the two top concentrations (2733 and 4957 ppm) at the 8 hour harvest time and actual increases in number of aberrations increased at these points with and without activation; a dose relationship was not apparent. This study has been reviewed and a DER generated (again, no Document No. has been assigned and does not appear to have a final sign-off). The review states this is an unacceptable study for several reasons: i) the positive control for activated conditions was inadequate, ii) no cytotoxicity found, and iii) the positive increases should be followed-up. It is the opinion of this reviewer that with the positive responses seen without activation and at levels without apparent cytotoxicity, this study needs to be upgraded to acceptable. The activity seen is consistent with other information on phosphine (see below). If the registrant wishes to perform an additional assay to address the three points above, the OPP will look at the results. For now, however, this is an acceptable positive study for assessment purposes and would satisfy the category of structural chromosomal aberrations for mutagenicity testing.

Other studies

A study measuring chromosomal aberration frequencies in cultured whole blood lymphocytes taken from rats after an in vivo inhalation exposure has been submitted to the Office of Toxic Substances in an 8(e) submission (#8EHQ-0291-1188). Rats were exposed to phosphine concentrations up to 25 ppm for 6 hours. Blood was obtained via cardiac puncture and lymphocytes cultured for cytogenetic analysis. Reproducible, significant increases in aberrations were found in male rats, but not females. In addition, complex aberrations which are rarely seen in unexposed young adult rats were found. This provides evidence that phosphine is capable

of inducing chromosomal events after an in vivo exposure.

In a published study (Garry et al., Science, 246: 251-255, 1989), fumigant applicators were examined for cytogenetic events after exposure to phosphine and/or other pesticides and fumigants. Briefly, in vitro cytogenetic studies using cultured human lymphocytes were first performed and suggested that exposure to phosphine slightly increases chromosomal damage over controls, primarily due to induction of gaps and deletions. Since these results were found at low concentrations of phosphine, it was expected that phosphine may induce chromosomal alterations at low concentrations in vivo. A cohort of professional fumigant applicators was identified and studies were performed on lymphocyte preparations. Samples were taken during the fumigating season. Again, an increase in total aberrations excluding gaps (includes deletions, breaks and other aberrations, e.g. rings and acentric fragments) over matched controls was found. This suggested that phosphine exposure was associated with increased chromosomal aberrations. No increase in sister chromatid exchanges was observed. Samples taken several weeks to months after the fumigating season indicated that the induction of aberrations dropped to similar levels as matched controls. Therefore, on a gross examination of the chromosomes, aberrations did not appear to be persistent long after initial exposure(s). However, this is consistent with the known half-lives of circulating lymphocytes of up to 2 weeks (although a small percentage of lymphocytes can persist for 9 months or more).

The above results were obtained from cytological examination of unbanded chromosome preparations. The finding of most concern comes from results obtained from banded chromosome preparations and analysis. Most of the applicators examined (11 out of 12 reported) had stable chromosome rearrangements (translocations and inversions) in at least one of their cells that was examined. This was in contrast to the matched controls (10 subjects) in which only 2 subjects had one translocation each. The numbers from the report are: 16 rearrangements in a total of 1200 cells (1.3%) examined from exposed applicators versus 2 rearrangements in a total of 1000 cells (0.20%) from controls. Since these types of chromosomal events are fairly rare (the controls here are not outside the usual reported frequencies), empirically, there appears to be an association with increased stable chromosomal rearrangements and phosphine exposure.

What exactly are the biological ramifications of these stable rearrangements is not certain. The report implies that further studies are needed to establish biologic significance. More recent analysis of rearrangement sites on the chromosomes of exposed applicators indicates that some of the translocations occur in a non-random manner (in Garry et al., Cancer Epidemiology, Biomarkers & Prevention, 1: 287-291, 1992). The sites of translocations appear to correlate in many instances with fragile sites, many of

which are known to be associated with oncogenes. An independent NCI study (Alavanja et al., JNCI 78: 247-252, 1987) suggest from mortality data that employees in the grain industry may be at elevated risk for lymphatic and hematopoietic cancers, particularly non-Hodgkins lymphoma. Some of the fragile sites in this study may be correlated with the incidence of this cancer.

Conclusions and recommendations

Overall, the evidence demonstrates that exposure to phosphine can induce clastogenic events, both in vitro and in vivo. While the induced elevations of chromosomal events in humans cannot be exactly tied to phosphine exposure since the applicators were also exposed to other chemicals, the rat inhalation study indicates that phosphine can induce such events in intact organisms after an inhalation exposure. This provides considerable support for the very probable association with phosphine exposure in humans and chromosomal events.

Based on this evidence, further mutagenicity testing is required and these requirements need to placed into the Data Call-In (DCI) for aluminum and magnesium phosphide. These are enumerated below:

1. A study to satisfy the other genotoxic effects category is required. An **in vivo/in vitro unscheduled DNA synthesis (UDS) assay** is the assay of choice. Rats are exposed via inhalation to phosphine gas and the hepatocytes taken after exposure to assay for UDS.

2. Based on the positive studies associating clastogenic effects in vivo in both rats and humans with phosphine exposure, there is a mutagenicity heritable concern that needs addressed where the germ cells are the target cells being assayed. A **rat dominant lethal study** with inhalation exposure is required.

Once the results of these assays are submitted, an assessment of all the pertinent data will be performed to ascertain if additional mutagenicity testing is still required, e.g. a quantitative test for heritable effects. This is consistent with the Subdivision F guideline for mutagenicity testing of pesticide chemicals (EPA-540/09-91-122, NTIS Publication No. PB91-158394) which supports the Part 158 Testing Requirements for the OPP (CFR 40 Part 158).