Recommendation from the Scientific Committee on Occupational Exposure Limits for hexamethylphosphoramide

SCOEL/SUM/156
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8-hour TWA	:	not feasible to derive a health-based limit (see "*Recommendation*")
STEL (15 mins)	:	not feasible to derive a health-based limit (see "*Recommendation*")
Notation	:	"Skin" notation
SCOEL carcinogen group:		B (genotoxic carcinogen, for which the existence of a threshold cannot be sufficiently supported at present)

Substance:

Hexamethylphosphoramide

Structural formula:
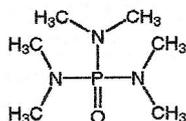
Identity and Properties:

Chemical name: Hexamethylphosphoric triamide
CAS No: 680-31-9
Empirical formula: $C_6H_{18}N_3OP$
Molecular mass: 179.2
Synonyms: Hexamethylphosphortriamide, (HMPA)
Melting point: 7°C
Boiling point: 233°C
Vapour pressure: 4Pa at 20°C
Conversion factor: 1 ppm = 7.33 mg/m³; 1 mg/m³ = 0.136 ppm

This summary document is mainly based on the documentations of IARC (1999) and DFG (2004), supplemented by a literature research of SCOEL.

1. Occurrence and use

Hexamethylphosphoramide has been produced commercially in small quantities. It is/was used as a solvent for polymers, a selective solvent for gases and as a thermal and ultraviolet radiation degradation stabilizer in various polymers (IARC, 1977).

2. Health significance

Hexamethylphosphoramide is reasonably anticipated to be a human carcinogen based on sufficient evidence in experimental animals (IARC 1977, 1999, ROC 1985). When administered by inhalation, the compound induced nasal tumours in rats of both sexes. Nasal epidermoid carcinomas were the predominant type of tumours observed; however, other nasal tumours included adenoid squameous carcinomas, papillomas, transitional carcinomas, and adenocarcinomas. No adequate human studies of the relationship between exposure to the compound and human cancer have been reported (ROC 1985, IARC 1999).

2.1. Toxicokinetics/metabolism

When labelled hexamethylphosphoramide (HMPA) was given intraperitoneally to rats and mice, 70% of the label was excreted within 20 min the urine. The parent compound undergoes a sequence of oxidative N-demethylations to yield pentamethylphosphoramide (PMPA), tetramethylphosphoramide and trimethylphosphoramide (TriMPA). In-vitro studies with rat liver slices indicated that the oxidative demethylation processes were associated with the simultaneous formation of formaldehyde, *e.g. via* hydroxymethyle-PMPA (IARC 1999).
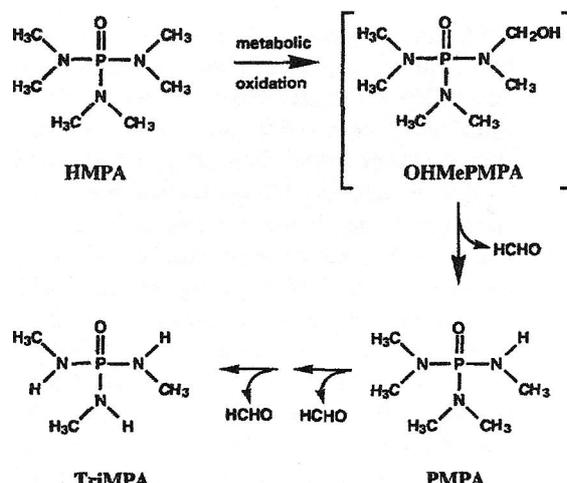
Thornton-Manning et al. (1997) demonstrated that in the rat nasal epithelium enzymes of the CYAP2A subfamily were present, which are able to demethylate hexamethylphosporamide. In human nasal tissues CYP2A6 mRNA was expressed. Human CYP2A6 metabolises hexamethylphosphoramide to formaldehyde. Later, Su et al. (2000) found CYP2A13 mRNA expressed in human nasal mucosa, from which the derived enzyme is even more potent to demethylate hexamethylphosphoramide than CYP2A6.

In essence, there is good evidence that hexamethylphosphoramide is locally metabolised (demethylated) to TriMPA in nasal epithelia of humans and experimental animals, under formation of 3 mol of formaldehyde per mol of the parent compound.

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2.2. Toxic effects

There are no data on effects in humans (IARC 1999).
The dermal LD_{50} in rabbits is given as 2600 mg/kg (DFG 2004). Upon application of hexamethylphosphoramide to rabbits under occlusion (6h/d, 5d/wk for 3 wk) at doses of 100 and 500 mg/kg dose-related weight loss, diarrhoea, anorexia and anuria, as well as CNS depression, mal-coordination and mortality were reported in both dose groups. Local skin effects were erythema and desquamation (Shott et al. 1971). This indicates a potential for skin absorption (DFG 2004).

Repeated inhalation of hexamethylphosphoramide by rats resulted in severe degenerative changes in renal convoluted tubules. Rats given this compound in the diet showed severe bronchiectasis and bronchopneumonia with areas of squamous metaplasia (IARC, 1977).

Inhalation by rats of 351 ppm for 15 min did not cause any decrease in respiratory rate (Gardner *et al.*, 1985). In the carcinogenicity experiment described below (Lee and Trochimowicz, 1982a), rhinitis, nasal epithelium degeneration, squamous metaplasia and dysplasia were observed in rats exposed to hexamethylphosphoramide by inhalation at concentrations of 50, 100, 400 or 4000 ppb for 6-24 months. No pathological lesions were found in the 10-ppb group after 24 months. Incidence of tracheitis, degeneration of the tracheobronchial epithelium and murine pneumonia was dose-related in the 100-, 400- and 4000-ppb groups. The ciliated cells were the most susceptible to hexamethyl–phosphoramide. Keratinized squamous metaplasia developed at 4000 ppb (Lee and Trochimowicz, 1982b,c).

2.3. Sensitisation

No data on sensitisation have been reported in humans or in experimental animals.

2.4. Genotoxicity

No data on genotoxicity in humans have been reported (IARC 1999).
A comprehensive review on genotoxicity assays conducted with hexamethylphosphoramide bas been presented by IARC (1999). For the experimental details, reference is made to this compilation. The data were summarised as follows.

Hexamethylphosphoramide gave negative results in several conventional assays for bacterial mutagenicity which employed commonly used *Salmonella typhimurium* strains in the presence or absence of exogenous metabolic activation systems. In one study, it gave positive results in two strains of *Escherichia coli* WP2, in the presence of an exogenous metabolic activation system (IARC 1999). In a more recent study, it was claimed to be mutagenic to several strains of *S. typhimurium,* in the presence of an exogenous metabolic activation system, when tested in a liquid suspension assay. Successive *N*-demethylation eliminates the mutagenic activity. This is interpreted in a way that the mutagenic activity of the compound is related to the metabolic release of formaldehyde (Sariff et al. 1997).

In *Drosophila melanogaster,* hexamethylphosphoramide gave positive results in tests for sex-linked recessive lethal mutations, heritable translocations, somatic mutation and differential survival in DNA repair-proficient and -deficient strains. In yeast, it induced mitotic gene conversion. Hexamethylphosphoramide induced micronuclei in newts treated *in vivo.*

In mammalian cells, it induced sister chromatid exchanges, micronuclei and gene mutations *in vitro.* In one study, it induced DNA-protein cross-links in rat nasal epithelial cells treated *in vitro.* In human cells *in vitro,* hexamethylphosphoramide induced micronuclei and sister chromatid exchanges.

In a single study *in vivo,* hexamethylphosphoramide induced sister chromatid exchanges in mouse bone marrow but not in mouse liver. In single studies *in vivo,* hexamethylphosphoramide did not induce chromosomal aberrations in mouse bone marrow, but did so in rat bone marrow. In several independent studies, it induced micronuclei in bone marrow of mice treated *in vivo.* Of two studies in which hexamethylphosphoramide was tested for induction of dominant lethal mutations in mice, one was positive and one was negative. It gave inconclusive or negative results in tests for abnormal sperm morphology in mice (IARC 1999).

2.5. Carcinogenicity

No data are reported on carcinogenic effects of hexamethylphosphoramide in humans (IARC 1999).

Hexamethylphosphoramide was tested for carcinogenicity in rats by inhalation; in this study it produced squamous-cell carcinomas of the nasal cavity. Experiments in rats by oral administration were considered inadequate (IARC, 1977).

Four groups of 120 male and 120 female Sprague-Dawley rats were exposed by inhalation to 0 (control), 50, 400 and 4000 ppb [0, 0.37, 2.9 and 29 mg/m^3] hexamethylphosphoramide vapour for 6 h per day an five days per week for periods ranging from nine months to two years. In an additional study, four groups of 100 male and 100 female rats were similarly exposed to 0, 10, *50* and 100 ppb [0, 73, 370 and 730 µg/m^3] atmospheres. Nasal tumours were first found after approximately seven months of exposure at 400 and 4000 ppb, after nine months at 100 ppb and after 12 months at 50 ppb. No exposure-related tumours were found at 10 ppb. Tumour incidences at 24 months were: 50 ppb, 15% (12 months of exposure) and 25% (24 months of exposure); 100 ppb, 19% (six months of exposure) and 56% (13 months of exposure); 400 ppb, 82% (10 months of exposure); 4000 ppb, 83% (nine months of exposure). Most tumours developed in the squamous or respiratory epithelium and nasal glands, all of which showed squamous metaplasia or dysplasia in the anterior nasal cavity. Exposure concentrations correlated with tumour incidence and latency, but not with tumour type. The total of 473 nasal tumours included 72% epidermoid carcinomas, 15% adenoid squamous carcinomas and 8% papillomas. Most tumours (59%) developed in the anterior nasal cavity and then progressed to the posterior nasal cavity (41%) (Lee & Trochimowicz, 1982a).

2.6. Reproductive toxicity

There are no reported data in humans (IARC 1999).

When rats were given daily doses of 200 mg/kg hexamethylphosphoramide on days 7-20 of gestation, no abnormalities were found in the offspring. The fertility of rats was not impaired by 10 mg/kg per day administered by gavage for 169 days (IARC, 1977).

Considerations of a mode of action

Hexamethylphosphoramide is a very potent experimental nasal carcinogen, which in a long-term bioassay has produced tumours in rats at a dose as low as 50 ppb (0.05 ppm). The metabolism of hexamethylphosphoramide in nasal tissues of rats leads to the production of formaldehyde via CYP-mediated N-demethylation (Ashby and Lefevre, 1982; Dahl and Hadley, 1983). Formaldehyde, like hexamethylphosphoramide, is carcinogenic to the rat nasal epithelium when given by inhalation and, like hexamethylphosphoramide, induces DNA-protein cross-links in target tissues (IARC, 1995; see also SCOEL/SUM/125). It is therefore plausible that metabolism of hexamethylphosphoramide within the nasal tissue leads to the local production of formaldehyde, which then forms DNA-protein cross-links (and possibly other DNA modifications), which in turn initiate carcinogenesis (Sarrif et al. 1997).

Studies of the pattern of mutagenicity of hexamethylphosphoramide in *D. melanogaster* have confirmed that this compound is a DNA cross-linking agent (Aguirrezabalaga *et al.*, 1995; Vogel and Natarajan, 1995). The local cross-linking activity of hexamethylphosphoramide is supported by the detection of DNA-protein cross-links in rat nasal epithelial cells treated *in vitro* (Kuykendall *et al.*, 1995). High-performance liquid chromatographic analysis of DNA extracted from flies injected with [^{14}C]hexamethylphosphoramide revealed no methylation at O^6 or $N7$ of guanine (Vogel *et al.*, 1985). This suggests that the formation of DNA adducts by hexamethylphosphoramide are not the result of methylation reactions (IARC 1999). However, formaldehyde is significantly more potent (about 60-fold) in forming DNA-protein cross-links than is hexamethylphosphoramide at equimolar concentrations, although the latter is substantially more carcinogenic (by nearly 100-fold) to the rat nasal epithelium than formaldehyde (Bogdanffy *et al.*, 1997). This suggests that DNA-protein cross-links alone may not be critical to the mechanism of the carcinogenicity of hexamethylphosphoramide. Based on studies of the mitogenic and tissue-damaging effects on the rat nasal epithelium of inhaled hexamethylphosphoramide (single exposures or five daily 1-h exposures at 3 ppm), Harman *et al.* (1997) postulated that its high carcinogenic potency could be explained on one hand by its ability to liberate formaldehyde intracellularly, and on the other hand by a stimulation mitogenesis in the absence of cytotoxicity. This is in clear contrast to formaldehyde, which appears to be carcinogenic only at doses that cause substantial tissue damage and which does not appear to be mitogenic at lower doses that do not damage the nasal epithelium (IARC, 1995; see also SCOEL/SUM/125). It is argued (Bogdanffy *et al.*, 1997; Harman *et al.*, 1997), that the stimulus for formaldehyde-induced cell proliferation is cytotoxicity, whereas for hexamethylphosphoramide it is mitogenesis. The efficiency with which promutagenic lesions induced by formaldehyde are converted to mutations would be low, since the cytotoxicity of epithelial cells would counteract the cell proliferation. In contrast, metabolites of hexamethylphosphoramide that accumulate in the tissue are considered to induce a mitogenic response such that the low levels of promutagenic lesions produced from formaldehyde would be more likely to be converted into mutations (IARC 1999).

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Recommendation

Hexamethylphosphoramide is a very powerful animal carcinogen. The target tissue is the nasal epithelium. Because of its limited industrial application, there are no data concerning toxicity or carcinogenicity in humans. Yet, based on the clear results in animals, it appears very likely that the compound should also be carcinogenic in humans. In a two-year inhalation bioassay in rats, nasal tumours were noted at 400 ppb (0.4 ppm) after 7 months, and at 50 ppb (0.05 ppm) after 12 months. No tumours were noted at 10 ppb (0.01 ppm). Studies have been conducted into the mode of action, related to nasal carcinogenesis (see above). There is some plausibility that the local liberation of formaldehyde (3 mol per mol hexamethyl–phosphoramide), together with a mitogenic effect of the compound itself or other metabolites, represent key issues for the nasal carcinogenicity (see the previous chapter). Such a mode of action could be expected to produce a dose-response curve that is non-linear in the low-dose region, *i.e.* below 10 ppb (Bogdanffy et al. 1997). However, the authors of the key contributions into the mechanism of hexamethylphosphoramide carcinogenicity have also clearly indicated themselves that further toxicokinetic and toxicodynamic studies are required for an understanding the carcinogenic susceptibility factors in human and rodent nasal tissues, and that the development of plausible modes of action lags behind the advancements made in dosimetry modelling (Bogdanffy et al. 1997).

Given this situation, a mode of action has been proposed for the apparently high carcinogenicity of hexamethylphosphoramide, which has some plausibility, but which still needs further support by experimentation and modelling. Clearly, the situation is more complicated and different compared to that of formaldehyde, for which SCOEL has proposed a health-based OEL (SCOEL/SUM/125).

In consequence, hexamethylphosphoramide is categorised in SCOEL carcinogen group B (Bolt and Huici-Montagud 2008) as a "genotoxic carcinogen, for which the existence of a threshold cannot be sufficiently supported at present". Hence, no health-based occupational exposure limit (TWA or STEL) can be deduced.

As clearly indicated by Bogdanffy et al. (1997), a reasonable quantitation of the carcinogenic risk for humans would require a more in-depth PB-PK (physiologically based pharmacokinetic) modelling, which is not available. Hence, development of a reasonable quantitative risk assessment is not possible at the present time.

Based on the results of the long-term inhalation bioassay, exposure concentrations at workplaces should be controlled and minimised, well below 10 ppb (0.01 ppm).

As systemic toxicity and lethality has been observed in rabbits after subacute dermal applications (see chapter "Toxic effects"), a "skin notation" is applied.

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