



Draft Assessment Report (DAR)

- public version -

**Initial risk assessment provided by the rapporteur Member State
Germany for the existing active substance**

CALCIUM PHOSPHIDE

**of the third stage (part B) of the review programme
referred to in Article 8(2) of Council Directive 91/414/EEC**

Volume 3, Annex B, part 2, B.6

October 2007

Annex B

Calcium phosphide

B-6: Toxicology and metabolism

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.6 Toxicology and metabolism

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

Metal phosphides in contact with moisture (GI tract) readily decompose to metal hydroxide and phosphine, the toxicological principle. Due to the decomposition by moisture other phosphides are regarded as adequate model compounds. Studies concerning absorption, distribution, metabolism and excretion of ingested zinc phosphide and phosphine are available. Once formed from the metal phosphide, phosphine is rapidly and completely excreted by exhalation or via urine after oxidation to hypophosphite or phosphite. The phosphine metabolites hypophosphite or phosphite are regarded as less toxic than phosphine itself. Due to the inorganic nature of the metal phosphides and its degradation products and their respective metabolites it is reasonable to assume that residues of these phosphides are expected to be minimal or non-existent. Following oral administration of zinc phosphide, [^{32}P] was rapidly absorbed from the gastrointestinal tract. Inhaled PH_3 is considered to be rapidly and quantitatively absorbed through the lungs. [^{32}P] was detectable in all organs and tissues, with temporary higher levels in liver and medulla oblongata. PH_3 is excreted as such with the expired air or, after metabolic oxidation, with the urine in the form of hypophosphite and phosphite. In the absence of experimental data, for dermal absorption of both calcium phosphide and PH_3 a default value of 10 %, based on expert judgement, was assumed.

Table B.6.1-1: Summary of toxicokinetic studies

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results	Reference
No guideline; Non-GLP	Oral	Rats, number, bw and sex not stated	Zinc phosphide 40 mg/kg bw (> LD_{50}) and lower dose (not specified), single application	Mortality↑ at high dose, PH_3 detectable in liver	Curry, A.S. et al. (1959) (TOX2002-163)
		Rats, sex not stated, 6 animals	Zinc phosphide 10 mg/rat, single application	Mortality↑, phosphide and PH_3 detectable in liver	
		Rats and guinea pigs, no further information given	No information given	Urinary excretion: main product is hypophosphite	

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results	Reference
No guideline, Non-GLP	Oral, subcutaneous, per rectum	Rattus norvegicus Berk, number, bw and sex not stated	Zinc phosphide, [^{32}P]-labelled 40 mg/kg bw	Oral application: After 6-8 h, ^{32}P was detectable in all organs and tissues with temporary higher levels in liver and medulla oblongata. Application per rectum: After 24 h ^{32}P was detectable in large intestine, arterial blood, liver and kidneys. Subcutaneous injection: After 24 h ^{32}P was detectable only around the point of injection.	Andreev, S.B. et al. (1958) (TOX2002- 165)
	Oral		Zinc phosphide, ^{32}P - and ^{65}Zn - labelled Sublethal, lethal, 2-, 3- and 4-fold lethal doses	The distribution of ^{32}P was similar to that in the above experiment. ^{65}Zn was found in all organs. The ratio of ^{32}P to ^{65}Zn was different in different tissues.	
No guideline, Non-GLP	oral	Human	Unknown quantity of Phostoxin tablets	Residues post mortem in stomach, blood, liver	Chan, L.T.F. (1983) (TOX98-50056)
Not applicable	Inhalation			Inhaled PH_3 is considered to be readily absorbed through the lungs, excretion with urine as hypophosphite and phosphite and via lungs as PH_3	WHO (1988) (TOX2005-1201)

Report: Curry, A.S. et al (1959): Absorption of zinc phosphide particles;
Nature 184, 642 – 643 (TOX2002-163)

Guidelines: No

Deviations: Not applicable

GLP: No

Acceptability: The study is considered to be supplementary.

Materials and Methods:

Zinc phosphide(^{32}P -labelled); batch no.: not stated; purity: no data; suspension of zinc phosphide (^{32}P -labelled) in commercially available evaporated milk were fed to adult 250g rats (gender unspecified) at a dose considered to be in excess of the LD_{50} (40 mg/kg bw), at 10 mg/kg bw and at lower unspecified doses. Subsequently, the determination of phosphine and phosphide in liver tissue was performed: Phosphine was flushed from the suspensions of the homogenised livers in water (cooled) by a stream of carbon dioxide, and any evolving PH_3 gas was collected by passing through a filter paper soaked with silver nitrate (replaced at half-hour intervals), resulting in [^{32}P]-silver phosphide, which was determined by β -scintillation counting. Next, the analysis for any 'unreacted' phosphide present was performed by acidification, again with collection of silver phosphide.

Findings:

In animals that were dosed in excess of the LD₅₀ and died shortly thereafter, phosphine was detectable in the livers. At lower doses (unspecified), when animals were killed more than 24 hours after treatment, no phosphine was detectable in the livers, but after addition of acid to the tissues, a very faint brown stain was obtained when the gases were passed through a filter soaked with methanolic silver nitrate. However, it was not possible to obtain a confirmation by a test for reduced phosphomolybdate blue. Animals no. 2 and 1 treated with 10 mg labelled zinc phosphide died less than 20 h after treatment and after about 22 h, respectively. The other four animals were killed 26 h after treatment. The livers of animals no. 1 and 2 were analysed separately, while the others were combined.

Conclusion:

Following oral administration of [³²P]-zinc phosphide to rats, the presence of phosphine/phosphide in the livers of animals was verified by β-scintillation counting. In those animals that obviously died from phosphine poisoning, phosphine and phosphide were both present in the liver. In contrast, the surviving animals had no detectable phosphine in their livers. However, upon acidification, the observed phosphine evolution indicates that zinc phosphide was present in their livers. Further experiments showed that the main urinary excretion product was hypophosphite, and no damage of the gastric and intestinal mucosa was found upon histological examination. The authors conclude that very fine zinc phosphide particles (< 0.1 micron) may pass through the intestinal wall of the GI tract to the blood stream. Whereas phosphine was no longer detectable in rats surviving the oral dosing, low levels of zinc phosphide were still present, indicating that this had been absorbed by surviving animals.

Report:	Andreev, S.B. et al. (1958): Some results of the use of tracer techniques in the study of plant protection; 2 nd Int. Conf. Peaceful Uses Atomic Energy 1958 (27), 85 – 92 (TOX2002-165)
Guidelines:	No
Deviations:	Not applicable
GLP:	No
Acceptability:	The study is considered to be supplementary.

Materials and methods:

Test material: Zinc phosphide [³²P]-labelled); batch no.: not stated; purity: no data.

The experiments were carried out on the grey rat, *Rattus norvegicus* Berk, to which were administered lethal doses (8 mg per 200 g live weight) of zinc phosphide (1) orally (pure substance), (2) subcutaneously (suspended in water) or (3) per rectum (suspended in water).

In the subsequent dissection, [³²P] content was analysed in samples of blood, liver, spleen, kidneys, lungs, muscles, bones, cortex and the medulla oblongata, stomach and intestine.

Findings:

Already 15 minutes after the oral administration of a lethal dose of zinc phosphide to rats, radioactivity is detectable in blood, liver and the anterior section of the intestinal tract. 30 minutes post dosing, radioactivity was also found in the posterior part of the intestine, as well as in spleen, kidneys and lungs, whereas the level in blood and livers had already considerably decreased. One hour p.a., radioactivity was widely distributed within the body, lacking only in brain, bone and muscle. At the same time, some radioactivity could already be recovered from urine. Upon death (usually within 6 – 8 hours p.a.), the radioactivity was present in all organs and tissues with a predominant accumulation in liver. Levels in stomach and intestine had considerably decreased, though still higher than in any other organ. Swelling of the stomach

and the small intestine was observed in poisoned animals, which was attributed to the presence of large amounts of PH_3 by analysis (silver phosphide precipitation). Radioactivity had also accumulated at the time of death in the medulla oblongata, correlating with disturbance of breathing and supporting the assumption that the toxicity of zinc phosphide is related to a disruption of respiratory function. For the elucidation whether phosphine is formed from zinc phosphide only in the stomach, zinc phosphide was also administered per rectum at the same dose level as above. 24 hours p.a., radioactivity was detectable in blood, liver and kidney, apart from the material present in the large intestine. It was not verified whether this radioactivity was in the form of phosphine or zinc phosphide. 24 hours after the subcutaneous administration, the radioactivity was detectable only at the site of injection, indicating that decomposition of the formation of mobile toxic compounds would not occur under these circumstances.

Conclusion:

Following oral administration of [^{32}P]-zinc phosphide to rats, radioactivity is rapidly absorbed and distributed. The limited absorption and diminished toxicity after administration per rectum demonstrates that hydrolysis in the acidic milieu of the stomach is the key process that mediates toxicity.

Report: WHO (1988): Environmental Health Criteria Document no. 73:
'Phosphine and selected metal phosphides', p. 50 (TOX2005-1201)

Guidelines: No

Deviations: Not applicable

GLP: No

Acceptability: The information is considered to be supplementary.

Metal phosphides are hydrolysed to phosphine and the corresponding metal cation. In rats, phosphine that is not excreted in the expired air is oxidised and appears in the urine, chiefly as hypophosphite and phosphite. An unidentified metabolite, detectable by paper chromatography and distinct from pyrophosphate and meta-phosphate, was reported. The fact that phosphine is incompletely oxidised; and the proportion of an administered dose that is eliminated as expired phosphine increases with the dose suggest that the oxidative pathway is slow.

Report: Chan, L. T. F. et al. (1983): Phosphine analysis in post mortem specimens following ingestion of aluminium phosphide; Journal of Analytical Toxicology, Vol. 7, July/August 1983 (TOX98-50056)

Guidelines: No guideline

Deviations: Not applicable

GLP: No

Acceptability: The study is considered to be supplementary.

Phosphide was detected in post mortem stomach, blood, and liver specimens from the body of a 27-year old man who died after ingestion of an unknown quantity of Phostoxin tablets (Degesch). These 3 g tablets, which contain aluminium phosphide as the active ingredient, slowly produce approximately 1 g phosphine when brought into contact with water. The phosphine was released from the samples after acid treatment and analysed by means of a headspace gas chromatographic technique using a nitrogen phosphorus detector.

B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2)

No acute oral toxicity study for calcium phosphide has been submitted by the applicant and no justification was given for that. However, there exist respective studies with other phosphides (summary see Table B.6.2-1). Metal phosphides in contact with moisture (GI tract) readily decompose to metal or calcium hydroxide and phosphine, the toxicological principle. Due to the decomposition by moisture other phosphides are regarded as adequate model compounds. Studies with aluminium phosphide and magnesium phosphide are available and are considered to be of high toxicity when administered orally to animals. Therefore calcium phosphide has to be classified as 'very toxic if swallowed' (T+; R 28). PH_3 , which is developed after contact of calcium phosphide with water by spontaneous hydrolysis of the phosphide, is very toxic by inhalation. According to Annex I to Directive 67/548/EEC classification and labelling of the gas is appropriate (T+; R 26), but calcium phosphide itself is like aluminium phosphide not classified with regard to inhalation toxicity.

No dermal toxicity study for calcium phosphide has been submitted. However, regarding calcium phosphide no higher acute dermal toxicity than observed in aluminium phosphide e.g. is expected (LD_{50} 460 – 900 mg/kg bw). Therefore, for calcium phosphide classification as 'harmful in contact with skin' (Xn; R 21) is required.

No skin irritation study for calcium phosphide has been submitted. However, calcium phosphide reacts like aluminium and zinc phosphide. For both substances no irritation was noted after application to the skin of rabbits. Therefore, for calcium phosphide no classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is required, too.

No eye irritation study for calcium phosphide has been submitted. However, studies for aluminium and zinc phosphide revealed no eye irritation potential. Therefore, for calcium phosphide no classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is required.

No skin sensitisation study has been presented using calcium phosphide. However, the study for zinc phosphide revealed no skin sensitisation potential. Therefore, calcium phosphide is considered to be not a sensitising substance, too, and classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is not required.

Table B.6.2-1: Overview on the acute toxicity of metal phosphides

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value LD ₅₀ /LC ₅₀	Risk Phrase Remarks	Reference
Acute oral toxicity. Similar to OECD 401 Non-GLP	Rat, Wistar albino 5M+5F	Aluminium phosphide 7.94-8.92-10.0- 11.2 mg/kg bw	LD ₅₀ M+F: 8.7 mg/kg bw	R 28	Sterner, W., Stiglic, A. (1977) (TOX2006- 981)
Acute oral toxicity OPPTS 870.1100	Rat Wistar, 3M + 3F	Aluminium phosphide 5 mg/kg bw	LD ₅₀ M+F: \approx 5 mg/kg bw	R 28	Stephen F. (2000) (TOX2006- 210)
Acute oral toxicity. Similar to OECD 401, Non-GLP	Rat Wistar, 5 M + 5 F	Aluminium phosphide 0-6-9- 13.5 mg/kg bw	LD ₅₀ : 9 mg/kg bw	R 28	Joshi M. (1998) (TOX2006- 211)
Acute oral toxicity. OECD 401	Mouse, NMRI/HAN Bö 5M+5F	Aluminium phosphide 6.81-10.0-14.7- 21.5 mg/kg bw	LD ₅₀ M+F: 14.8 mg/kg bw	R 28	Leuschner, F. (1992) (TOX2005- 308)
Acute oral toxicity. Similar to OECD 401, Non-GLP	Mouse CD, 5M + 5F	Aluminium phosphide 0, 9, 11.2, 14 mg/kg bw	LD ₅₀ M+F: 12 mg/kg bw	R 28	Joshi M. (1998) (TOX2006- 212)
Acute oral toxicity No guideline Non-GLP	Rat	Zinc phosphide No dose levels mentioned	LD ₅₀ : 12 mg/kg bw	R 28	Lewis, R. J. (Eds.) (2000) (TOX2006- 19):
Acute oral toxicity No guideline Non-GLP	Rat, CFT-Wistar albino 10F	Zinc phosphide 21, 28, 38, 51, 68 mg/kg bw	LD ₅₀ F: 43 – 56 mg/kg bw	R 25	Krishnakumari , M. et al. (1979) (TOX2002- 167)
Acute oral toxicity No guideline Non-GLP	Deer mice 6 animals	Zinc phosphide	ALD: 42 mg/kg bw	Not acceptable	Schafer, E.W., Bowles, W.A. (1985) (TOX2002- 168)
Acute oral toxicity. No guideline Non-GLP	Rat, Wistar albino 5M+5F	Magnesium phosphide 0.897, 1.0, 1.13, 1.26 g/kg bw total amount	LD ₅₀ M: 20.7 mg/kg bw F: 10.4 mg/kg bw M+F: 11.2 mg/kg bw	R 28	Sterner, W., Chibanguza, G. (1980) (TOX2000- 89)
Acute dermal toxicity. OECD 402	Rat, Wistar albino 5M+5F	Aluminium phosphide 500-1000-2000 mg/kg bw	LD ₅₀ M+F: 900 mg/kg bw	R 21	Dickhaus, S., Heisler, E. (1987) (TOX2000- 93)
Acute dermal toxicity OPPTS 870.1200	Rat Wistar, 5 F/each level + 5 M/highest level	Aluminium phosphide 0-280-420-630 mg/kg bw	LD ₅₀ : 461.2 mg/kg bw	R 21	Stephen F. (2000) (TOX2006- 213)

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value LD ₅₀ /LC ₅₀	Risk Phrase Remarks	Reference
Acute dermal toxicity. No guideline Non-GLP	Rat Wistar, 5M+5F	Aluminium phosphide 0-637.7-1275-2550 mg/kg bw	LD ₅₀ : 901 mg/kg bw	R 21	Joshi M. (1998) (TOX2006-214)
Acute dermal toxicity Comparable to 92/69/EEC, B3	Rat, Wistar albino 5M+5F	Zinc phosphide 1000-2000-4000 mg/kg bw	LD ₅₀ M+F: 1000 mg/kg bw	R 21	Dickhaus, S., Heisler, E. (1980) (TOX2002-172)
Acute dermal toxicity Comparable to 92/69/EEC, B3	Rat, Wistar albino 5M+5F	Zinc phosphide 100-200-400-1000 mg/kg bw	LD ₅₀ M+F: 525 mg/kg bw	R 21	Dickhaus, S., Heisler, E. (1980) (TOX2002-173)
Acute inhalation toxicity. No guideline, non GLP	Rat Wistar, 5M+5F	Aluminium phosphide 0-15.4-26-47 ppm	LC ₅₀ : 34.6 ppm (0.048 mg/L)	R 26	Roy, B.C. (1998) (TOX2006-215)
Inhalation whole body 6 h exposure, US EPA	Rat Fisher 344	PH ₃ 2.4-4.9-11 ppm	LC ₅₀ M+F: >11 ppm equivalent to > 0.015 mg/L or > 0.675 mg/kg bw	R 26	Newton, P.E. (1989) (TOX97-51198)
Acute inhalation toxicity, whole body, 1 h exposure Similar to OECD 403 Non-GLP	Rat, Slc:SD 10M+10F	PH ₃ , developed from magnesium phosphide 150-165-182-200-220-242 ppm	LC ₅₀ : 204/179 ppm (M/F) equivalent to ⁽²⁾ : 0.29/0.25 mg/L air (M/F) or ⁽³⁾ 12.9/11.4 mg/kg bw (M/F)	(R 26, PH ₃) ⁽¹⁾	Shimizu, Y. et al. (1982) (TOX2005-280)
Acute skin irritation, Partly OECD 404	Rabbit, White New Zealand, 5 (sex not mentioned)	Aluminium phosphide 0.5 g/animal	Not irritating	None	Dickhaus, S., Heisler, E. (1987) (TOX2000-94)
Acute skin irritation. No guideline, non-GLP	Rabbit, White New Zealand, 3M+3F	Aluminium phosphide 0.5 g/animal	Non-irritant	None	Joshi M. (1998) (TOX2006-216)
Acute skin irritation, OECD 404	Rabbit, New Zealand White 3M	Zinc phosphide 0.5 g/animal	Not irritating	None	Brunt, P. (2001) (TOX2005-168)
Acute eye irritation OECD 405	Rabbit White, New Zealand 6 (sex not mentioned)	Aluminium phosphide 0.1 g/animal	Non-irritant (washed out 30 seconds after application)	Study design not suitable	Dickhaus, S., Heisler, E. (1987) (TOX2000-95)
Acute eye irritation. No guideline, non-GLP	Rabbit, White New Zealand, 3M + 3F	Aluminium phosphide 1 mg/animal	Not acceptable	Study design not suitable	Joshi, M. (1998) (TOX2006-217)

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value LD ₅₀ /LC ₅₀	Risk Phrase Remarks	Reference
Acute eye irritation OECD 405	Rabbit, White New Zealand, 2M+1F	Zinc phosphide 0.1 mL/animal	Non-irritant	None	Brunt, P. (2001) (TOX2005- 171)
Skin sensitisation OECD 406	Albino Guinea Pig (10M)	Zinc phosphide	Non-sensitising	None	Brunt, P. (2001) (TOX2002- 179)

- (1) PH₃ was included into Annex I to Directive 67/548/EEC with R 26, whereas the different phosphides were not classified for inhalation toxicity.
- (2) 1 ppm PH₃ is equivalent to 1.41 µg/L air, density of pure PH₃ (20 °C): (34 g/mol)/(24.1 L/mol) = 1.41 g/L
- (3) Assuming an hourly respiratory volume (rat) of 45 L/(h kg bw)

B.6.2.1 Acute oral toxicity

No acute oral toxicity study on animals for calcium phosphide has been submitted by the applicant and no reason was given for that. Only a study in rats for a preparation has been provided (see B.6.11). However, metal phosphides like aluminium phosphide and magnesium phosphide are considered to be of high toxicity when administered orally to animals. Therefore calcium phosphide has to be classified as 'very toxic if swallowed' (T+; R 28), too.

Report: Sterner, W., Stiglic, A. (1977): Acute oral toxicity of 'Aluminium phosphide' in rats, International Bio-Research, Hannover, Germany; unpublished report no. 0-0-51-77, 01/1977 (TOX2006-981)

Guidelines: No

Deviations: Exceeded application volume.

GLP: No

Acceptability: The study is considered to be acceptable.

Materials and methods:

A single oral dose of aluminium phosphide (technical grade) was given to 5 male and 5 female SPF-Wistar rats/dose group by stomach tube. The body weight of the rats was 140-175 g prior to dosing. In order to apply aluminium phosphide, it was mixed with vaseline to yield a concentration of 1 %. Before use this preparation was suspended in anhydrous olive oil to obtain a final concentration of 0.1 % (no information is given whether this refers to w/v or v/v). The doses administered were 7.94, 8.92, 10.00, and 11.2 mg aluminium phosphide/kg bw. Different doses were applied using different volumes of the test suspension described above. The recommended application volume of 10 mL/kg bw was exceeded. Clinical signs, mortality and body weights were recorded. All surviving animals were sacrificed after 7 days. Macroscopic examinations of all animals were performed and gross pathologic changes were reported.

Findings:

For detailed results see Table B.6.2-2. At a dose of 7.94 mg aluminium phosphide/kg bw, 1/5 males and 1/5 females died within day 1, at 8.92 mg/kg bw 3/5 males and 3/5 females died, and at 10.0 mg/kg bw and above all animals died. Survivors recovered by day 2 p.a.. No effect

on body weight gain was observed among survivors throughout the post-exposure period. The oral LD₅₀ for aluminium phosphide was calculated to be 8.7 mg/kg bw for both sexes.

Table B.6.2-2: Acute oral toxicity of aluminium phosphide in rats

Dose [mg/kg bw]	Number of dead / number of inves- tigated	Time of death (range)	Observations
7.94	1/5 females 1/5 males	Day 1 Day 1	decreased motor activity, coordination disturbance, abnormal body posture, decreased grip- and limb tone, decreased reflex excitability, tremor, exophthalmus and diarrhea; body weight gain of survivors was unaffected; necropsy findings: swollen liver observed in all animals and serious redness of intestinal mucous membrane in animals that died <i>post applicationem</i>
8.92	3/5 females 3/5 males	Day 1 Day 1	the same symptoms as described above but more pronounced; body weight gain of survivors was unaffected; necropsy findings: swollen liver observed in all animals and serious redness of intestinal mucous membrane in animals that died <i>post applicationem</i>
10.0	5/5 females 5/5 males	Day 1 Day 1	the same symptoms as described above but more pronounced; necropsy findings: swollen liver observed in all animals and serious redness of intestinal mucous membrane in animals that died <i>post applicationem</i>
11.2	5/5 females 5/5 males	Day 1 Day 1	the same symptoms as described above but more pronounced; necropsy findings: swollen liver observed in all animals and serious redness of intestinal mucous membrane in animals that died <i>post applicationem</i>
LD ₅₀ value males + females:			8.7 (8.2 – 9.3) mg/kg bw

Conclusion:

The LD₅₀ for aluminium phosphide in albino rats was calculated as 8.70 (8.17 – 9.27) mg/kg bw for males and females by oral administration.

Classification as ‘very toxic if swallowed’ (R 28) is required according to Directive 2001/59/EC (adaptation of 67/548/EEC).

Report

Stephen, F. (2000): Acute oral toxicity study of aluminium phosphide technical in rats (fixed dose method), JAI Research Foundation (JRF), Gujarat, India, JRF study No. 2565, date 17.10. 2000 (TOX2006-210)

Guidelines:

US EPA FIFRA Guideline OPPTS 870.1100 and OECD 420

Deviations:

Homogeneity and stability of the dose were not determined. However, the dose was prepared freshly prior to dosing. Batch number of test substance was not reported.

GLP:

Yes (laboratory certified by The Netherlands authorities)

Acceptability:

The study is considered to be supplementary.

Materials and methods:

Following a range-finding preliminary test a single female died within 1 hour after dosing at 500 and at 50 mg/kg bw, animals were assigned: 5 male and 5 female Wistar rats (breeding facilities at JAI Research Foundation, India; 11 weeks at time of dosing) were given a single dose of 5 mg/kg bw of aluminium phosphide technical (85.65 % pure; batch not reported) by gavage. The test substance was administered in peanut oil at a volume of 10 mL/kg bw. Animals were observed for gross toxicity, behavioural changes and/or mortality at approximately 1, 2, 3 and 6 hours after dosing and twice daily for the remainder of the 14-day study. Body weights were recorded at day 0 prior to dosing, 7 and 14. On day 14, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes. The data did not warrant statistical analysis.

Findings:

Details are provided in Table B.6.2-3. Four males died within 3 hours after dosing. All females survived.

Table B.6.2-3: Acute oral toxicity of aluminium phosphide in rats

Dose [mg/kg bw]	Males		Females	
	Mortality	Time of death	Mortality	Time of death
5	4/5	1-3 hour (day 1)	0/5	--
LD ₅₀ [mg/kg bw]	~ 5 (both sexes combined)			

Clinical signs on the day of dosing were tremors. No signs were observed on subsequent days up to the end of the observation period.

All surviving animals gained weight 7 and 14 days following dosing.

Necropsy: Vascular/emphysematous changes in lungs and mottling of liver were recorded in all early deaths. Vascular alteration in lungs, liver, kidneys and adrenals were recorded in surviving animals at study termination.

Despite the homogeneity and stability of the dose not being determined, the study is nevertheless acceptable. The dose was prepared freshly prior to dosing. The deviation did not compromise the acceptability of the study.

Conclusion:

The acute oral LD₅₀ of aluminium phosphide technical in rats was found to be ~ 5 mg/kg bw for both sexes combined. According to Commission Directive 2001/59/EC (adaptation to 67/548/EEC), classification as 'very toxic if swallowed' is required (R 28).

Report:

Joshi, M. (1998): Acute oral toxicity test of aluminium phosphide technical in rats, JAI Research Foundation (JRF), Gujarat, India
JRF study No. 361, 27.10.1998 (TOX2006-211)

Guidelines:

Gaitonde subcommittee, Central Insecticide Board (CIB), India

Deviations:

Concentration, homogeneity and stability of the dose preparations were not determined. However, the doses were prepared freshly prior to dosing. Observation period limited to 7 days. However, all deaths occurred shortly after dosing. Purity of test substance was not indicated. Batch number and purity of test substance were not reported. Age of the animals was not reported. Environmental conditions like air changes and photoperiod were not reported. Temperature of the experimental animal room was higher during the

study (27 – 28 °C) instead of the recommended 20 ± 3 °C.
GLP: No
Acceptability: The study is considered to be supplementary.

Materials and Methods:

Following an overnight fast (17 hours), groups of 5 male and 5 female Wistar rats (breeding facilities at JAI Research Foundation, India) were given single doses of 0, 6, 9 or 13.5 mg/kg bw of aluminium phosphide technical (purity/batch, age of animals were not reported) was administered in peanut oil at a volume of 10 mL/kg bw. Animals were observed for gross toxicity, behavioural changes and/or mortality at approximately 1, 2, and 3 hours after dosing and at least once daily for the remainder of the 7-day study. Body weights were recorded at day 0 (prior to dosing) and 7. On day 7, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Findings

Details are provided in Table B.6.2-4. The early deaths occurred within 3 hours after dosing.

Table B.6.2-4: Acute oral toxicity of aluminium phosphide in rats

Dose [mg/kg bw]	Males		Females	
	Mortality	Time of death	Mortality	Time of death
0	0/5	--	0/5	--
6	0/5	--	0/5	--
9	4/5	1-3 hour (day 1)	1/5	1-3 hour (day 1)
13.5	4/5	1-3 hour (day 1)	3/5	1-3 hour (day 1)
LD ₅₀ [mg/kg bw]	9 (both sexes combined)			

Clinical signs in treated animals on the day of dosing were toe walking, abdominal breathing, polyurea, piloerection and salivation. No signs were observed on subsequent days up to the end of the observation period.

All surviving animals gained weight following dosing.

Necropsy: No external abnormalities were detected. Gross changes observed in the viscera were mainly vascular in nature and considered to be associated with terminal sacrifice procedures.

Conclusion:

The acute oral LD₅₀ of aluminium phosphide technical in rats was found to be 9 mg/kg bw for both sexes combined. According to Commission Directive 2001/59/EC (adaptation to 67/548/EEC), classification as ‘very toxic if swallowed’ is required (R 28).

Report: Leuschner, F. (1992): Acute toxicity study of aluminium phosphide by oral administration to NMRI mice, LPT, Hamburg, Germany; unpublished report no. 7129/92, 15.06.1992; dates of experimental work: 02.03.1992 to 19.03.1992 (TOX2005-308)
Guidelines: OECD guideline 401
Deviations: Application volume of 20 mL/kg bw instead of 10 mL/kg bw
GLP: Yes
Acceptability: The study is considered to be acceptable.

Materials and methods:

Test material: Aluminium phosphide; batch no. 2009; purity: 82 %. The study was performed with 20 male and 20 female mice/ NMRI/HAN Bö (SPF) with initial weight of 18-23 g and an age of 30-35 days. The test material was ground before and suspended in sesame oil DAB 10 and was applied to 5 animals/sex and dose group by gavage. The doses administered were 6.81, 10.0, 14.7 and 21.5 mg/kg bw in an application volume of 20 mL/kg bw (according to the OECD guideline 401 max. application volume is 10 mL/kg bw). Post-exposure period was 14 days. During this time clinical signs, mortality and body weights were recorded. At the end of the experiment all surviving animals were sacrificed. Macroscopic examinations of these animals as well as of those which had died during the study were performed and gross pathologic changes, if any, were reported. The LD₅₀ was calculated by regression analysis.

Findings:

Under the test conditions applied no effects could be detected at a dose of 6.81 mg aluminium phosphide/kg bw. First intolerance reactions observed were reduced motility, ataxia and dyspnoea (see Table B.6.2-5). These clinical signs occurred at a dose of 10.0 mg aluminium phosphide/kg bw. Two males and two females died within 4 hours after dosing at 14.7 mg aluminium phosphide/kg bw, whereas all animals died during the first 2 hours after treatment at 21.5 mg/kg bw. They died in abdominal position with clonic convulsions or in coma. Body weight gain was not affected throughout the post exposure period nor were there any pathological findings. The LD₅₀ was calculated to be 14.8 mg/kg bw for both sexes.

Table B.6.2-5: Acute oral toxicity for aluminium phosphide in mice

Dose [mg/kg bw]	Number of dead / number of investigated	Time of death (range)	Observations
6.81	0/5 females 0/5 males	- -	-
10.0	0/5 females 0/5 males	- -	reduced motility, ataxia and dyspnoea; bw development and post mortem examinations revealed no effect of treatment
14.7	2/5 females 2/5 males	Day 1 Day 1	reduced motility, ataxia and dyspnoea; all survivors recovered by day 2, bw development (survivors) and post mortem examinations revealed no effect of treatment
21.5	5/5 females 5/5 males	Day 1 Day 1	reduced motility, ataxia and dyspnoea; post mortem examinations revealed no effect of treatment
LD ₅₀ value	Males + females: 14.8 mg/kg bw		

Conclusion:

The LD₅₀ for aluminium phosphide in mice was calculated as 14.8 mg/kg bw for males and females by oral administration.

Classification as 'very toxic if swallowed' (R 28) is required according to Directive 2001/59/EC (adaptation of 67/548/EEC).

Report:	Joshi, M. (1998): Acute oral toxicity test of aluminium phosphide technical in mice, JAI Research Foundation (JRF), Gujarat, India JRF study No. 362, 27.10.1998 (TOX2006-212)
Guidelines:	Gaitonde subcommittee, Central Insecticide Board (CIB), India
Deviations:	Concentration, homogeneity and stability of the dose preparations were not determined. However, the doses were prepared freshly prior to dosing. Observation period was limited to 7 days. Purity/batch of test substance was not mentioned. Age of the animals is not reported. Environmental conditions like air changes and photoperiod were not reported. Temperature of the experimental animal room was higher during the study (27 – 28 °C) instead of the recommended 20 ± 3 °C.
GLP:	No
Acceptability:	The study is considered to be supplementary.

Materials and Methods:

Following an overnight fast (15 hours), groups of 5 male and 5 female CD mice (breeding facilities at JAI Research Foundation, India) were given single doses of 0, 9, 11.2 or 14 mg/kg bw of aluminium phosphide technical (purity/batch not mentioned) by gavage. The test substance was administered in peanut oil at a volume of 10 mL/kg bw. Animals were observed for gross toxicity, behavioural changes and/or mortality at approximately 1, 2, and 3 hours after dosing and at least once daily for the remainder of the 7-day study. Body weights were recorded at day 0 (prior to dosing) and 7. On day 7, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Findings:

Details are provided in Table B.6.2-6. The early deaths occurred within 3 hours after dosing.

Table B.6.2-6: Acute oral toxicity of aluminium phosphide in mice

Dose [mg/kg bw]	Males		Females	
	Mortality	Time of death	Mortality	Time of death
0	0/5	--	0/5	--
9	0/5	--	0/5	--
11.2	1/5	1-3 hour (day 1)	1/5	1-3 hour (day 1)
14	4/5	1-3 hour (day 1)	5/5	1-3 hour (day 1)
LD ₅₀ [mg/kg bw]	12 (both sexes combined)			

Clinical signs in treated animals on the day of dosing were lethargy, abdominal breathing, polyurea, and diarrhoea. No signs were observed on subsequent days up to the end of the observation period.

All surviving animals gained weight following dosing.

Necropsy: No external abnormalities were detected. Gross changes observed in the viscera were mainly vascular in nature and considered to be associated with terminal sacrifice procedures.

Conclusion:

The acute oral LD₅₀ of aluminium phosphide technical in mice was found to be 12 mg/kg bw for both sexes combined. According to Commission Directive 2001/59/EC (adaptation to 67/548/EEC), classification as 'very toxic if swallowed' (R 28) is required).

Report: Lewis, R. J. (Eds.) (2000): Zinc phosphide; In: Dangerous Properties of Industrial Materials, 10th Ed. Vol. 3, 3519, published.

Guidelines: No

Deviations: Insufficient documentation

GLP: No

Acceptability: The publication is considered to be supplementary.

Materials and Methods:

In this review publication no further information were given.

Findings/Conclusion:

The acute oral toxicity LD₅₀ for zinc phosphide cited in this review publication is 12 mg/kg bw in rats. Therefore classification as 'very toxic if swallowed' (T+; R 28) is required.

Report: Krishnakumari, M.K.et al. (1980): Toxicity and rodenticidal potency of zinc phosphide; Bull. Environ. Contam. Toxicol. 25, 153-159 (TOX2002-166)
and
Krishnakumari, M.K. et al. (1979): Evaluation of acute oral toxicity of zinc phosphide in Rattus norvegicus (albino); Pesticides 13, 33-35. (TOX2002-167)

Guidelines: No

Deviations: Only female rats were used. Age, source, acclimatisation period environmental conditions were not mentioned. Concentration, homogeneity, stability and batch number of the samples were not determined.

GLP: No

Acceptability: The study is considered to be supplementary.

Materials and methods:

Groups of ten partially starved female albino rats (CFT-Wistar, 200-250 g) received dose levels of 21, 28, 38, 51 and 68 mg/kg of zinc phosphide (source A, purity 87 %) in peanut oil by gavage. Control rats were given only peanut oil. No details on the dose regime were stated for zinc phosphide from the sources B (83 %), C (94 %) and D (99.9 %). Animals were observed for clinical signs and mortality during the 4-week observation period. Food consumption and body weight were recorded regularly. Before autopsy, blood samples were collected and haematological parameters were determined (including haemoglobin, RBC, WBC, PCV, ESR and differential counts). At necropsy, organ weights (i.e. liver, kidney, heart, spleen, lungs and ovary) and pathological/histopathological investigations were performed.

Findings:

Percent mortality increased from 10 to 60 % with increasing dose levels. Deaths occurred in general within 40 hours after treatment. Results of the determination of LD₅₀ and LD₉₀ values by Probit analysis are presented in Table B.6.2-7.

Clinical signs such as heavy breathing, ataxia, restlessness, coma and paralysis of hind limbs appeared within 5 minutes, and convulsions occurred prior to deaths. Food consumption was affected in higher doses, but body weight was not significantly affected. Histopathological investigations revealed slight haemorrhages in glomeruli and tubules of kidneys of some rats

in different doses (21, 28 and 38 mg/kg bw), and mild cellular infiltrations around bronchioles of the lungs were found.

Table B.6.2-7: Acute oral toxicity of four different zinc phosphide samples in female rats

Samples of Zinc phosphide	Dose levels	% Mortality (hrs)*	LD ₅₀ [mg/kg bw]	95% confidence limits [mg/kg bw]	LD ₉₀ [mg/kg bw]
A (87.0%)	0 21 28 38 51 68	0 10 (18) 10 (18) 20 (18) 60 (18-40) 60 (18-64)	54	33 – 120	120
B (83.0 %)			56	---	120
C (94.0 %)			44	---	100
D (99.9 %)			43	---	97

*) average death time

Conclusions:

Following oral administration of different zinc phosphide suspensions LD₅₀ values in the range of 43 to 56 mg/kg bw were obtained.

Report:

Schafer, E.W., Bowles, W.A. (1985): Acute oral toxicity and repellency of 933 chemicals to house mice and deer mice; Arch. Environ. Contam. Toxicol. 14, 111-129. (TOX2002-168)

Guidelines:

No

Deviations:

Insufficient documentation

GLP:

No

Acceptability:

The study is considered to be not acceptable.

Materials and Methods

Zinc phosphide obtained from a commercial supplier (not specified) was tested in acute oral toxicity tests with wild-trapped deer mice (sex not specified). Six animals were treated with a graduated dosage scale and the ALD (Approximate Lethal Dose) was determined. One single animal per dose level received the test compound by gavage and was subsequently observed for 3 days. Each succeeding dose level was 50 % higher than the preceding level and continued until mortality occurred.

Findings:

Apart from the ALD value (42.0 mg/kg bw) no further findings have been presented.

Conclusions:

The integrity and quality of the study is not assignable due to insufficient documentation. However, the determined ALD (Approximate Lethal Dose) value is 42.0 mg/kg.

Further studies

Tkadlek and Gattermann (TOX 2002-170). (1993) investigated circadian changes in susceptibility of voles and golden hamsters to zinc phosphide. No significant results were obtained.

Nakata et al (TOX2002-171). (1993) investigated acute symptoms caused by 1 percent zinc phosphide pellets in the gray red-backed vole.

Report: Sterner, W. and Chibanguza, G. (1980): Acute oral toxicity of 1 % magnesiumphosphid in vaseline in rats, IBR International Bio-Research, Hannover, Germany; report no. 1-4-666-79, 02/80 (TOX2000-89)

Guidelines: No

Deviations: Exceeded application volume. Batch not mentioned.

GLP: No

Acceptability: The study is considered to be supplementary.

Materials and methods:

A single oral dose of magnesium phosphide was given to 5 male and 5 female albino rats (SPF-Wistar, Winkelmann, Paderborn). In order to apply magnesium phosphide was mixed with vaseline to yield a concentration of 1 %. Before use this preparation was suspended in oleum arachidis. The test sample had a pH-value of 6. The dose levels were 0.897, 1.0, 1.13 and 1.26 g/kg bw (total amount). Different doses were applied using different volumes of the test suspension described above. The recommended application volume of 10 mL/kg bw was exceeded. All animals were observed closely for gross signs of systemic toxicity and mortalities at frequent intervals on the day of treatment and at least once daily thereafter for a total period of 14 days.

Gross necropsies were performed on the animals that died. At the end of the 14 day period the surviving rats were weighed, sacrificed and gross necropsies were performed. For statistical analysis of the mortality data the system of Probit analysis by Finney D.Y. was used.

Findings:

In this study the test material induced decreased activity, abnormal body posture, reduced pain reaction, reduced grip- and limb- tone and piloerection. The mortalities occurred during 24 h after administration (see Table B.6.2-8). Redness of the gastrointestinal mucous membrane was observed. No effect on body weight gain was observed among survivors throughout the post exposure period. Necropsies performed on the surviving animals at termination exhibited no gross pathological findings. The LD₅₀ values were calculated by the Probit analysis and are shown in Table B.6.2-9.

Table B.6.2-8: Acute oral toxicity of magnesium phosphide in rats

Dose [g/kg bw] Total amount	Number of dead / number of investigated	Time of death
0.897	0/5 females 0/5 males	Day 1 Day 1
1.0	2/5 females 0/5 males	Day 1 Day 1
1.13	4/5 females 3/5 males	Day 1 Day 1
1.26	5/5 females 3/5 males	Day 1 Day 1

Table B.6.2-9: Calculated LD₅₀ of magnesium phosphide in rats

	Total amount	Active substance
Male	2.07 g/kg bw	20.7 mg/kg bw
Female	1.04 g/kg bw	10.4 mg/kg bw
Male + Female	1.12 g/kg bw	11.2 mg/kg bw

Conclusion:

The oral LD₅₀ values for albino rats were calculated as 10.4 mg/kg bw for females, 20.7 mg/kg bw for males and 11.2 mg/kg bw for combined sexes.

Classification as 'very toxic if swallowed' (R 28) is required according to Directive 2001/59/EC (adaptation of 67/548/EEC).

B.6.2.2 Acute percutaneous toxicity

No dermal toxicity study with calcium phosphide has been submitted.

The following justification for non-submission a skin irritation study with calcium phosphide has been submitted by the applicant (Köhler, U (1999). TOX2000-110):

'Calcium phosphide is dry granular solid which decomposes very rapidly on contact with water to produce highly toxic gas phosphine. Hydrolysis of calcium phosphide on the skin would lead to the evolution of gaseous phosphine, which could then be absorbed by inhalation. As result it is not considered valid to measure the acute dermal toxicity of calcium phosphide, since contact with dermal moisture will result in rapid decomposition and evolution of phosphine and calcium dihydroxide which will invalidate any attempt at estimation. Dermal absorption of calcium phosphide and phosphine is regarded to be insignificant (see: WHO Environmental Health Criteria No. 73, Phosphine and Selected Metal Phosphides, page 49 (1988). The acute dermal LD₅₀ for zinc phosphide in rats has been reported: LD₅₀ 24 hours 2000 mg/kg bw, LD₅₀ 14 days 1000 mg/kg bw.

Please refer to the studies on the acute dermal toxicity for zinc phosphide in rats'.

Comment by the RMS :

The justification is accepted. Furthermore, the acute dermal toxicity of metal phosphides differ. Aluminium phosphide is more toxic than zinc phosphide e.g : The LD₅₀ values were calculated to be 460 – 900 mg/kg bw and therefore classification as 'harmful in contact with skin' (Xn; R 21) is required for both substances. Regarding calcium phosphide no higher acute dermal toxicity than observed in aluminium phosphide is expected. Consequently, calcium phosphide should be classified as 'harmful in contact with skin' (Xn; R 21).

Report:

Dickhaus, S., Heisler, E. (1987): Acute toxicological study on compound aluminium phosphide after dermal application to the rat, pharmatox, Hanover, Germany, unpublished report no. 1-4-142-87, 09/1987 (TOX2000-93)

Guidelines:

Although the test facility claims that this study was conducted according to OECD guideline 404, it complies with OECD guideline 402

Deviations:

Neither purity or batch of test material were mentioned.

GLP: Yes

Acceptability: The study is considered to be supplementary.

Materials and methods:

A single dermal dose of aluminium phosphide (purity/batch not mentioned) was applied to the clipped skin of 5 male and 5 female SPF-Wistar rats/dose group under occlusive conditions. Dose levels of 500, 1000 and 2000 mg aluminium phosphide/kg bw were tested. Initial body weights of the rats were 206 – 230 g for males and 202 – 212 g for females, respectively; no information is given about the age of the animals. Prior to application solid granules of aluminium phosphide were minced. Deviating from applicant's study summary it remains unclear from the original study report, whether the test substance was applied as a powder or whether it had been moistened before. No information is provided about the size of the skin area treated with aluminium phosphide. The skin was exposed to the substance for 24 hours. Afterwards residual test substance was removed from the skin using a wet-warm towel and the animals were observed for deaths, clinical signs and body weight gain for 14 days. At the end of the study the remaining rats were sacrificed and all animals were examined macroscopically for pathological findings. The method of calculating LD₅₀ was not mentioned but it was performed in combination with Gauss' integral method.

Findings:

No death occurred at 500 mg aluminium phosphide/kg bw; while at a dose of 1000 mg/kg bw, 3/5 males and 3/5 females died and all animals died at 2000 mg/kg bw. No information is given concerning recovery of survivors. Body weight gain was gradually reduced at increasing aluminium phosphide dose levels. The dermal LD₅₀ of aluminium phosphide was calculated to be 1520 mg/kg bw (24 hours) or 900 mg/kg bw (day 14) for both sexes by the applicant. Assuming that aluminium phosphide had been applied to the skin as crystalline granules (see above) it would not have adhered just as well on the skin as if a fluid had been applied (apart from the fact that phosphine gas would have been developed simultaneously), i. e. higher doses would have been needed in the first way to yield the same effects as in the latter and a lower LD₅₀ would be expected. Nevertheless, it is unlikely that this would have led to a different classification.

Conclusion:

The dermal LD₅₀ of aluminium phosphide was calculated to be 1520 mg/kg bw (24 hours) or 900 mg/kg bw (day 14) for both sexes in rats. According to Directive 2001/59/EC (adaptation of 67/548/EEC) classification as 'harmful in contact with skin' (R 21) is required.

Report: Stephen, F. (2000): Acute dermal toxicity study of aluminium phosphide technical in rats. JAI Research Foundation (JRF), Gujarat, India, JRF study No. 2566, date 23.10.2000 (TOX2006-213)

Guidelines: OPPTS 870.1200

Deviations: Concentration, homogeneity and stability of the dose preparations were not determined. However, the doses were prepared freshly prior to dosing. Batch of test substance was not reported. Environmental conditions like air changes and photoperiod were not reported. Temperature of the experimental animal room was higher during the study (27-28 °C) instead of the recommended 20 ± 3 °C.

GLP: Yes (laboratory certified by The Netherlands authorities)

Acceptability: The study is considered to be supplementary.

Materials and Methods:

Following a range-finding preliminary test with 1 male and 1 female per group in which mortalities of 0 %, 50 % and 100 % were observed at dose levels of 250, 500 and 1000 mg/kg bw, respectively, rats (Wistar, breeding facilities at JAI Research Foundation, India) were assigned to the test groups (see Table B.6.2-10). One day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal. The clipped area accounted approximately 10 % of each animal's body surface. The test substance (purity 85.65 %) was administered as a single occluded dermal application and was applied moistened with peanut oil. After an exposure period of 24 hours, the occlusion was removed and residual test material was removed with dry cotton and tissue paper. Animals were observed for gross toxicity, behavioural changes and/or mortality at approximately 30 minutes, 1, 2, 3 and 5 hours after dermal application and twice daily for the remainder of the 14-day study. Body weights were recorded at day 0 (prior to dosing), 7 and 14. On day 14, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Findings:

Details are provided in Table B.6.2-10. All early deaths occurred within 48 hours after dermal application.

Table B.6.2-10: Acute dermal toxicity of aluminium phosphide in rats

	Females		Males	
Dose [mg/kg bw]	Mortality	Time of death	Mortality	Time of death
0	--	--	0/5	--
280	--		0/5	--
420	--		2/5	5 hours 30 min (day 1)
630	4/5	4 x 48 hours	4/5	2 x 5 hours 30 min (day 1) 2 x 48 hours
LD ₅₀ [mg/kg bw]	461.2 (both sexes combined)			

Clinical signs in treated animals on the day of dosing and the day after dosing were lethargy, tremors, abdominal breathing and piloerection. No signs were observed on subsequent days up to the end of the observation period.

All surviving animals showed normal body weight gain following dosing.

Necropsy: No external abnormalities were detected. Vascular/inflammatory alterations in lungs, mottling of liver and hemorrhagic contents in stomach and small intestinal segments were noted in premature decedents. Gross changes observed in the viscera were considered to be associated with terminal sacrifice procedures.

Conclusion:

The acute dermal LD₅₀ of aluminium phosphide technical in rats was found to be 461.2 mg/kg bw for both sexes combined. According to Commission Directive 2001/59/EC (adaptation to 67/548/EEC), classification as 'harmful in contact with skin' is required (R 21).

Report:

Joshi, M. (1998): Acute dermal toxicity test of aluminium phosphide technical in rats, JAI Research Foundation (JRF), Gujarat, India, JRF study No. 363, 27.10. 1998 (TOX2006-214)

Guidelines:

Gaitonde subcommittee, Central Insecticide Board (CIB), India

Deviations: Concentration, homogeneity and stability of the dose preparations were not determined. However, the doses were prepared freshly prior to dosing. Observation period limited to 7 days. Purity of test substance not mentioned. Age of the animals is not reported. Environmental conditions like air changes and photoperiod were not reported. Temperature of the experimental animal room was higher during the study (27 – 28 °C) instead of the recommended 20 ± 3 °C.

GLP: No

Acceptability: The study is considered to be supplementary.

Materials and Methods:

Wistar rats (breeding facilities at JAI Research Foundation, India) were assigned to the test groups (see Table B.6.2-11). One day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal. The clipped area accounted not less than 10 % of each animal's body surface. The test substance was administered as a single occluded dermal application and was applied moistened with peanut oil. After an exposure period of 24 hours, the occlusion was removed and residual test material was removed with wet cotton. Animals were observed for gross toxicity, behavioural changes and/or mortality at approximately 1, 2, and 3 hours on the day of dosing and once daily for the remainder of the 7-day study. Body weights were recorded at day 0 (prior to dosing) and 7. On day 7, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Findings:

Details are provided in Table B.6.2-11. All early deaths occurred on the day of dosing.

Table B.6.2-11: Acute dermal toxicity of aluminium phosphide

Dose [mg/kg bw]	Females		Males	
	Mortality	Time of death	Mortality	Time of death
0	0/5	--	0/5	--
637.5	1/5	1-3 hour (day 1)	1/5	1-3 hour (day 1)
1275	4/5	24 hour (day 1)	4/5	24 hour (day 1)
2550	5/5	2 x 1-3 hour (day 1) 3 x 24 hour (day 1)	5/5	1 x 1-3 hour (day 1) 4 x 24 hour (day 1)
LD ₅₀ [mg/kg bw]	901 (both sexes combined)			

Clinical signs in treated animals on the day of dosing and the day after dosing were lethargy, abdominal breathing, nasal irritation, polyurea, and diarrhoea. No signs were observed on subsequent days up to the end of the observation period.

All surviving animals showed normal body weight gain following dosing.

Necropsy: No external abnormalities were detected. Gross changes observed in the viscera were considered to be associated with terminal sacrifice procedures.

Conclusion:

The acute dermal LD₅₀ of aluminium phosphide technical in rats was found to be 901 mg/kg bw for both sexes combined. According to Commission Directive 2001/59/EC (adaptation to 67/548/EEC), classification as 'harmful in contact with skin' (R 21) is required.

Report:	Dickhaus, S. and Heister, E. (1980): Akute Toxizitätsprüfung von der Substanz ‚Zinkphosphid‘ nach dermalen Applikation an der Ratte, report no. 1-4-258-80 (TOX2002-172)
Guidelines:	In consideration of ‘Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics’, Division of Pharmacology, FDA 1959.
Deviations:	Batch number, purity not mentioned. No identification if the active substance or a product has been tested. Different information regarding the observation period for mortality were given.
GLP:	No
Acceptability:	The study is considered to be supplementary.

Material and methods:

Zinc phosphid (80 %), batch number and purity not mentioned. SPF Wistar rats (Winkelmann, Paderborn, Germany), 5 males and 5 females per group. The dose levels were 1000, 2000 and 4000 mg/kg bw, administered dermal as a slurry to the shaved, intact skin (occlusive, 24 hours).

Clinical symptoms and behaviour were observed for 14 days. Regarding mortality observation different periods were mentioned: 24 and 48 hours, 7 days (in study protocol); 24 hours, 7 and 14 days (in materials/methods, results, summary). At day 14 animals were killed for necropsy. Determination of LD₅₀ by Probit analysis according to Lichtfield & Wilcoxon.

Findings:

Animals showed ataxia, sedation and tremor. Back posture, apathy and coma were seen in animals which died. Animals of the low dose group gained body weight, and surviving animals of the mid dose group maintained their body weight. At necropsy no remarkable compound related macroscopic changes in main tissues and organs were observed. Haemorrhagic infiltrations were seen at the application sites. The mortality rates (assuming 24 hours, 7 and 14 days were correct) are shown in Table B.6.2-12.

Table B.6.2-12: Mortality rates after 24 hours, 7 and 14 days in rats

Dose mg/kg bw	Mortality rates					
	24 hours		7 days		14 days	
	Males	Females	Males	Females	Males	Females
1000	0/5	0/5	0/5	0/5	0/5	0/5
2000	2/5	4/5	4/5	4/5	4/5	4/5
4000	4/5	4/5	5/5	5/5	5/5	5/5

Conclusion:

The dermal (intact skin) LD₅₀ of zinc phosphide in rats was calculated to be for 24 hours 2000 mg/kg bw (1750 – 2300) or for 14 days 1000 mg/kg bw (909 – 11001) for both sexes. Classification as ‘harmful in contact with skin’ (R 21) is required for magnesium phosphide according to Directive 2001/59/EC (adaptation of 67/548/EEC).

Report:	Dickhaus, S. and Heister, E. (1980): Akute Toxizitätsprüfung von der Substanz 'Zinkphosphid' nach dermalen Applikation an der skarifizierten Haut der Ratte, report no.1-4-258a-80 (TOX2002-173)
Guidelines:	No
Deviations:	Batch number, purity not mentioned. No indication if the active substance or a product has been tested. Different information regarding the observation period for mortality was given
GLP:	No
Acceptability:	The study is considered to be supplementary.

Material and methods:

Zinc phosphid (80 %), batch number and purity were not mentioned. SPF Wistar rats (Winkelmann, Paderborn, Germany), 5 males and 5 females per group. The dose levels were 100, 200, 400 and 1000 mg/kg bw, administered dermal as a slurry to the scarified skin (occlusive, 24 hours).

Clinical symptoms and behaviour were observed for 14 days. Regarding mortality observation period different information were given: 24 and 48 hours, 7 days (study protocol); 24 hours, 7 and 14 days (materials/methods, results, summary). At day 14 animals were killed for necropsy. Determination of LD₅₀ by Probit analysis according to Lichtfield & Wilcoxon.

Findings:

Animals showed sedation, apathy, tremor, back posture and coma prior to death. All surviving animals gained body weight throughout the 14 day observation period, but a dose related decrease of weight gain with increasing dose levels was observed. At necropsy no remarkable compound related macroscopic changes in main tissues and organs were observed. Haemorrhagic infiltrations were seen at the application sites. The mortality rates (assuming 24 hours, 7 and 14 days were correct) are shown in Table B.6.2-13.

Table B.6.2-13: Mortality rates after 24 hours, 7 and 14 days in rats

Dose mg/kg bw	Mortality rates					
	24 hours		48 hours		7 days	
	Males	Females	Males	Females	Males	Females
100	0/5	0/5	0/5	0/5	0/5	0/5
200	0/5	0/5	0/5	0/5	0/5	0/5
400	1/5	0/5	1/5	1/5	1/5	2/5
1000	4/5	3/5	4/5	4/5	5/5	5/5

Conclusion:

The dermal (scarified skin) LD₅₀ of zinc phosphide in rats was calculated to be 775 mg/kg bw (24 hours) or 525 mg/kg bw (14 days) for both sexes. According to Directive 2001/59/EC (adaptation of 67/548/EEC) classification as 'harmful in contact with skin' (R21) is required.

B.6.2.3 Acute inhalation toxicity

Report:	Roy, B. C. (1998): Acute inhalation toxicity test of aluminium phosphide technical in rats, JAI Research Foundation (JRF), Gujarat, India JRF study No. 366 30.11.1998 (TOX2006-215)
Guidelines:	Gaitonde subcommittee, Central Insecticide Board (CIB), India
Deviations:	The purity and batch number of test substance were not mentioned. Observation period limited to 7 days.
GLP:	No
Acceptability:	The study is considered to be supplementary.

Materials and Methods:

Wistar rats (breeding facilities at JAI Research Foundation, India) were observed approximately hourly during the 4-hour exposure period. Thereafter clinical observations and mortality checks were conducted once daily. Individual body weights were determined before exposure and day 7. On day 7, surviving animals were necropsied and examined for gross pathological changes.

A head only exposure chamber was used. The exposure unit was made up of stainless steel with 20 port-holes to accommodate rat exposure tubes. A dust generator system was used to generate the test aerosols. The breathing zone phosphine concentrations were monitored at hourly intervals.

Findings:

Details are provided in Table B.6.2-14. All early deaths occurred during the 4-hour inhalation exposure period.

Table B.6.2-14: Mortality results for aluminium phosphide in rat inhalation LC₅₀ study

Dose [ppm (mg/L)]	Males		Females	
	Mortality	Time of death	Mortality	Time of death
0	0/5	--	0/5	--
15.4 (0.0215)*	0/5	--	1/5	1-4 hours (day 1) [°]
26 (0.0364)*	2/5	1-4 hours (day 1) [°]	1/5	1-4 hours (day 1) [°]
47 (0.0658)*	3/5	1-4 hours (day 1) [°]	4/5	1-4 hours (day 1) [°]
LC ₅₀ [ppm (mg/L)]	34.6 ppm (0.048 mg/L); 4h (both sexes combined)			

* Conversion factor (1 ppm = 0.0014 mg/L) was based on the density of phosphine being ca 1.4 kg/m³ at 1 bar and 20 °C

[°] during exposure

Nasal irritation was noted for treated animals during the exposure period. Surviving animals gained weight during the observation period. Necropsy: There were no significant external abnormalities. Early deaths showed vascular changes in lung, liver, spleen and adrenals, and the highest severity was noted in the high dose group. Terminal sacrifice revealed vascular changes in lung and kidney.

Conclusion:

The acute inhalation LC₅₀ of phosphine liberated from aluminium phosphide technical in rats was found to be 34.6 ppm (0.048 mg/L) for both sexes combined. According to

Commission Directive 2001/59/EC (adaptation to 67/548/EEC), classification as ‘very toxic by inhalation’ is required (R 26).

Report: Newton, P.E. (1989): An acute inhalation toxicity study of phosphine (PH₃) in the rat. Metal phosphide task force. Biodynamics, Inc. Mettlers Road, East Millstone, New Jersey 08875, USA, report no.: 87-8029, 5 September 1989, dates of experimental work: 30 June 1988 to 15 July 1988 (TOX97-51198)
and
Newton, P. E. et al (1993): Inhalation toxicity of phosphine in the rat: acute, subchronic, and developmental. Inhalation Toxicol. 5, 223-229, 1993 (TOX2002-824)

Guidelines: US EPA 81-3 (1984)

Deviations: Period of exposure was 6 hours. Observations post exposure were carried out weekly.

GLP: Yes (US EPA)

Acceptability: The study is considered to be supplementary.

Materials and Methods:

Groups of 15 male and 15 female Fisher 344 rats (supplied by Charles River Breeding Laboratories, Inc. Kingston, New York 12484) were exposed to phosphine in a whole body inhalation exposure system for 6 hours to 0, 2.5, 5 and 10 ppm of the test substance (mean analytical exposure level were 0, 2.4, 4.9 and 11 ppm). Exposure atmospheres of phosphine were generated from compressed gas mixtures of 1 % phosphine in nitrogen which was metered into the air intake turret of the exposure chambers. The concentration of phosphine in the test atmosphere was analysed by gas chromatography with samples taken at least four times during the exposure period. Nominal concentration was calculated by monitoring the flow of phosphine through the chamber and dividing the resultant value by the flow of air during the exposure. This value was converted in parts per million. Chamber airflow, temperature and relative humidity were monitored every 30 minutes. The main exposure parameters were summarised in Table B.6.2-15:

Table B.6.2-15: Main exposure parameters

Exposure period	6 hours
Exposure chamber volume	1000 L
Chamber air flow	200 L/minute
Air changes	12/hour
Chamber temperature range	17.2 - 23.9 °C (63 – 75° F)
Relative humidity range	45 – 63 %
Atmosphere equilibration time T99	23 minutes
Target concentration	0, 2.5, 5 and 10 ppm
Nominal concentration	0, 2.2, 4.2 and 7.2 ppm
Mean analysed concentration	0, 2.4, 4.9 and 11 ppm

All animals were observed at about fifteen minute intervals during the exposure period, and upon removal from the chamber (half an hour after exposure was completed). After exposure, 5 animals per sex per group were killed and examined and specified tissues were weighed and retained. Histopathology was performed on brain, heart, kidney, liver and lungs. Detailed

observations were recorded for remaining animals weekly during the 14 days post exposure. Individual body weights were recorded immediately prior to exposure and on day 8 and 14 post exposure. At study termination all remaining animals were necropsied. Body weights were analysed statistically. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control. A statistical test for trend in the exposure levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used. The test for equal variance (Bartlett's) was conducted at the 1 %, two-sided risk level. All other statistical tests were conducted at the 5 % and 1 %, two-sided risk level.

Findings:

There were no mortalities. A red or mucoid discharge was observed in some animals in all treated group, however most animals showed no clinical signs. During the 14 day recovery period, these observations disappeared. No body weight differences related to treatment were observed. Necropsy revealed no treatment related findings. No treatment related changes were seen in the animals sacrificed on day 1, therefore tissues from animals sacrificed on day 15 were not subjected to microscopic examinations. The acute (6 hour) inhalation LC₅₀ of phosphine was > 11 ppm (> 0.015 mg/L) in female and male rats. This LC₅₀ value compares well with the following 4-hour inhalation values: LC₅₀ rat (m): 11 ppm (0.016 mg/L) (Waritz and Brown, 1975, TOX2002-176), LC₅₀ rat (f): 32 ppm (0.045 mg/L), 5.2-hrs exposure, (Muthu et al., 1980, TOX2006-171); LC₅₀ rat (f): 45.3 ppm (0.064 mg/L), 7.4-hrs exposure (Muthu et al., 1980, TOX2006-171); LC₅₀ mouse (m): 26.5-33.4 ppm (0.037 – 0.047 mg/L) (Omae et al., 1996, TOX2002-174).

Conclusion:

The acute (6 hour) inhalation LC₅₀ of phosphine was > 11 ppm (> 0.015 mg/L) in female and male rats. Sum up all available LC₅₀ values for phosphine, classification as 'very toxic by inhalation' (R 26) is required according to Directive EU 2001/59/EC (adaptation of 67/548/EEC).

Report:

Shimizu, Y., Ogawa, Y., Tokiwa, K. (1982): Acute inhalation toxicity testing of hydrogen phosphide in rats, Nomura Research Institute, Japan, unpublished report no. NRI 82-7489, 31.05.82, dates of experimental work: January 1982 – May 1982 (TOX2005-280)

Guidelines:

Test is similar to the OECD guideline 403

Deviations:

Exposure period was 1 hour instead of 4 hours, no data for concentration measurement

GLP:

No

Acceptability:

The study is considered to be supplementary.

Materials and methods:

Hydrogen phosphide, generated by the reaction of magnesium phosphide and distilled water; batch No. 82016. 10 male and 10 female SD rats per dose group were exposed to PH₃ gas

generated by the spontaneous reaction of magnesium phosphide and distilled water in a whole-body exposure experiment. The animals were exposed to 150, 165, 182, 200, 220 and 242 ppm PH_3 over a period of 1 hour. Based on different amounts of magnesium phosphide put into the exposure chamber the resulting PH_3 concentrations were calculated in advance. The notifier describes that the concentrations have been confirmed by measurements during the test, but data are not reported. Clinical signs, deaths and body weight gains were recorded; the post-exposure observation period was 14 days. Dead and surviving animals were examined pathologically.

Findings:

The general symptoms in male and female rats were almost the same (see

Table B.6.2-17). During the period up to approximately thirty minutes after the initiation of exposure, so-called body-extending behaviour (stretching, clinging to the wire mesh walls) and face-washing action were observed. Thereafter, during the period from forty minutes after the initiation of the exposure to the end of the exposure some rats suddenly started running about then lay prone, or lay prone after falling into convulsions. Death took place in four to five minutes at the earliest and in seven hours at the latest after the onset of these symptoms. Macroscopic observations revealed nothing abnormal. Some detailed data are reported in Table B.6.2-16. Concentrations given in this table represent calculated values. At a concentration of 165 ppm PH_3 3/10 females died whereas 1/10 males died at 182 ppm. At the highest concentration used (242 ppm) no test animals survived. From the results presented the following LC_{50} were calculated: 204 ppm (confidence limits 195 – 213 ppm) for male rats and 179 ppm (confidence limits 170 – 188 ppm) for female rats. Body weight was reduced immediately after exposure. With the exception of the 220 ppm exposure group, body weight of male and female rats increased on the day after exposure but recovered to almost the same levels as before. Since according to Directive 2001/59/EC (adaptation of 67/548/EEC) limit concentrations for classification regarding inhalation toxicity are derived from 4 hour inhalation studies, it is not possible to decide offhand whether a classification is needed for magnesium phosphide or not. Newton et al. (1993, TOX2002-824) found out in a PH_3 inhalation study using Fischer 344 rats that deaths occurred around a concentration-time product of 180 ppm h, provided a threshold concentration of approximately 7 ppm is exceeded. These results confirmed earlier findings of Klimmer (1969, TOX96-52057) who reported a concentration-time product of 213 ppm h for male Wistar rats and similar products for cats, guinea pigs, rabbits and chickens. Furthermore, Muthu et al. (1980, TOX2006-171) tested PH_3 generated by hydrolysis of two different aluminium phosphide containing preparations for its inhalation toxicity in female albino CFT-Wistar rats. The LC_{50} for one of the products was 28 ppm at an exposure period of 5.2 hours and 33 ppm for the other product for an inhalation time of 7.4 hours. The appropriate concentration-time products are 146 ppm h and 244 ppm h, respectively which fit well in the concentration-time product range above. The lowest four-hour LC_{50} for phosphine in male rats (ChR-CD) that up to now has been reported was 11 ppm equivalent to 0.015 mg PH_3 /L air (The corresponding concentration-time product is 44 ppm h) (Waritz and Brown, 1975, TOX2002-176). According to the information given above, the LC_{50} of the study presented is converted into an LC_{50} referring to a 4-hour incubation time. Thus, the LC_{50} is 51 ppm for males and 44.8 ppm for females, corresponding to 0.072 mg PH_3 /L air and 0.063 mg PH_3 /L air, respectively. Since the toxic effect of magnesium phosphide is due to liberated PH_3 which itself is classified appropriately (i. e. T+; R 26 in the 29th ATP of Directive 67/548/EEC) no classification of magnesium phosphide is required regarding inhalation toxicity. Moreover, magnesium phosphide has already been inserted in Annex I of Directive 67/548/EEC without classification concerning this end point.

Table B.6.2-16: Weights of magnesium phosphide placed in the exposure chamber to obtain the desired hydrogen phosphide concentration

Weight of Mg_3P_2 (g)	0.441	0.485	0.535	0.588	0.647	0.711
Calculated Concentration (ppm)*	150	165	182	200	220	242
* 0.5 g PH_3 /g phosphide assumed; $MW(PH_3)$: 34 g/mol						

Table B.6.2-17: Acute Inhalation Toxicity

Dose [ppm], 1 h exposure	Number of dead / number of investigated	Time of death (range)	Observations
150	0/10 females 0/10 males	- -	None No necropsy findings
165	3/10 females 0/10 males	Day 1 -	None No necropsy findings
182	6/10 females 1/10 males	Day 1 Day 1	None among survivors; Tonic convulsions; run about prior to death No necropsy findings
200	10/10 females 4/10 males	Day 1 Day 1	None among survivors; Tonic convulsions; run about prior to death No necropsy findings
220	8/10 females 8/10 males	Day 1 Day 1	None among survivors; Tonic convulsions; run about prior to death No necropsy findings
242	10/10 females 10/10 males	Day 1 Day 1	Tonic convulsions; run about prior to death No necropsy findings
LC_{50} males (1 h): 204 ppm (confidence limits 195 – 213 ppm); 51 ppm deduced for 4 hrs corresponding to 0.072 mg PH_3 /L air LC_{50} females (1 h): 179 ppm (confidence limits 170 – 188 ppm); 44.8 ppm deduced for 4 hrs corresponding to 0.063 mg PH_3 /L air			

Conclusion:

For PH_3 , an LC_{50} , 1 h = 204 ppm (0.29 mg PH_3 /L air) and 179 ppm (0.25 mg PH_3 /L air) was calculated for males and females, respectively. After converting the present study into an LC_{50} referring to a 4 hour incubation time an LC_{50} = 51 ppm (0.072 mg PH_3 /L air) and 44.8 ppm (0.063 mg PH_3 /L air) was calculated for males and females, respectively.

B.6.2.4 Skin irritation

No skin irritation study with calcium phosphide has been submitted. Justification has been provided and accepted. The assessment of calcium phosphide is based on data for zinc and aluminium phosphide.

Report:

Dickhaus, S. and Heisler, E. (1987): Irritant effects of aluminium phosphide on intact skin of rabbits, pharmatox, Hannover, Germany; unpublished report no. 1-3-183-87, 10/1987 (TOX2000-94)

Guidelines:

Although the test facility claims that this study was conducted according to OECD guideline 406 it was similar to OECD guideline 404.

Deviations: Neither purity or batch of test material, age and sex of tested animals were mentioned. 24 hrs occlusive conditions, skin readings after 24 and 72 hrs.

GLP: Yes

Acceptability: The study is considered to be supplementary.

Materials and methods:

Test material: 0.5 g pulverised aluminium phosphide (no information about the purity is given; no batch number is mentioned) were deviating from the guideline applied to 2.5 cm² shaved skin of 5 (once 6 animals are mentioned in the study protocol, but results for 5 rabbits are presented only) white New Zealand rabbits (body weights were about 3.0 kg, no information about age or sex) under occlusive conditions for 24 hours (deviating from guideline). Skin readings were performed immediately after patch removal and 48 hours later (deviating from guideline). Grading of skin reactions was done according to Draize. The examination of the treated skin area was continued up to 7 days.

Findings:

For results see also Table B.6.2-18. Immediately after patch removal, i. e. after 24 hours of test substance application, all rabbits had oedema grade 1 which disappeared completely by the second reading (48 hours after removal of aluminium phosphide, i. e. 72 h after application). At no time point erythemas were recorded. Aluminium phosphide is slightly irritant to the skin but the average scores are well below the threshold for classification according to Directive 2001/59/EC. After 24 hours of contact all localisations at intact skin did show slight oedema, further 48 hours later there were no deviations at intact skin compared to normal skin.

Table B.6.2-18: Skin irritation study (average score of 5 animals investigated):

		Erythema	Oedema
Time after start of application	24 h	0	1
	72 h	0	0
Average score 24 h, 72 h		0	0.5
Reversibility:		-	Completely reversible

Conclusion:

No classification for skin irritation is required for aluminium phosphide according to Directive 2001/59/EC (adaptation of 67/548/EEC).

Report: Joshi, M. (1998): Primary skin irritation test of aluminium phosphide technical in rabbits, JAI Research Foundation (JRF), Gujarat, India, JRF study No. 364, 15.10.1998 (TOX2006-216).

Guidelines: Gaitonde subcommittee, Central Insecticide Board (CIB), India

Deviations: The exposure period was 24 hours instead of 4 hours. Test substance purity and batch number were not mentioned. The test substance was applied to intact and abraded skin. Temperature of the experimental animal room was higher during the study (28 - 30 °C) instead of the recommended 20 ± 3 °C. Environmental conditions like air changes and photoperiod were not reported. No information is given regarding supplier and age of animals.

GLP: No
Acceptability: The study is considered to be supplementary.

Materials and Methods:

On the day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal at four sites. Each clipped site was about 6 cm² in area and the sites served as control and test sites for intact and abraded skin. Abrasion was performed using tip of a sterilised hypodermic needle drawn across the skin repeatedly. The test material was applied, semi-occluded, as a single dermal administration to 3 male and 3 female New Zealand White rabbits. The application rate was 0.5 mg per animal. The test material was applied as a powder, on control sites 0.5 mL peanut oil was applied. The areas were covered with aluminium foil, gauze patch which was secured with non-irritating tape. After an exposure period of 24 hours, the occlusion was removed and residual test material was removed with soaked cotton. The test sites were examined for signs of erythema and oedema at 24, 48 and 72 hours following patch removal. Irritation was scored using the Draize scheme.

Findings:

Slight erythema was noted in four intact skin sites at 24 hours post treatment (see Table B.6.2-19). This had resolved by 48 hours. No other effect was seen. Abrasion of the skin caused no additional effects. The primary skin irritation index was calculated by the addition of averages of mean values for oedema and erythema at 24 and 72 hours. It was found to be 0.33 for the test substance.

Table B.6.2-19: Individual and mean skin irritation scores for intact skin (Draize scheme)

Animal No	Erythema						Oedema					
	1M	2M	3M	4F	5F	6F	1M	2M	3M	4F	5F	6F
after 24 h	1	0	1	1	0	1	0	0	0	0	0	0
after 48 h	0	0	0	0	0	0	0	0	0	0	0	0
after 72 h	0	0	0	0	0	0	0	0	0	0	0	0
mean score 24-72 h	0.33	0	0.33	0.33	0	0.33	0	0	0	0	0	0
	0.2						0.0					

Conclusion:

Aluminium phosphide technical induces only very slightly skin irritation reactions. Therefore, no classification for skin irritation is considered required according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC).

Report: Brunt, P. (2001): Zinc phosphide: Acute dermal irritation in the rabbit; Safepharm Laboratories, Derby, U.K., project No.: 1483/001, unpublished report, March 1, 2001 (TOX2005-168)
Guidelines: OECD Guideline 404 'Acute Dermal Irritation/Corrosion' (adopted 17 July 1992); Commission Directive 92/69/EEC Method B4 Acute Toxicity (Skin Irritation)
Deviations: None
GLP: Yes
Acceptability: The study is considered to be acceptable.

Materials and methods:

Zinc phosphide, batch no.: 2000-10-24, purity: 82 %. For 4 hours a quantity of 0.5 g test material (moistened with 0.4 mL distilled water) was given as a single semi-occluded application (2.5 x 2.5 cm) to the intact skin of three male New Zealand White rabbits (2.0 – 3.5 kg). Approximately one hour following removal of the patches, and 24, 48 and 72 hours later, test sites were examined for evidence of primary irritation. Evaluation was according to classification scheme of Draize.

Findings:

No evidence of skin irritation (erythema/eschar and oedema) was noted during the study. The primary irritation index was 0.0.

Conclusion:

The test material was classified as non-irritant according to the classification scheme of Draize. No classification is required according to the classification criteria of the EU Commission Directive 93/21 EEC.

B.6.2.5 Eye irritation

No eye irritation study with calcium phosphide has been submitted. However, justification has been provided and accepted. The assessment of calcium phosphide is based on studies for zinc and aluminium phosphide: No classification for eye irritation is considered required according Commission Directive 2001/59 EC (adaption to 76/548/EEC).

Report:	Dickhaus, S. and Heisler, E. (1987): Irritant Effects of aluminiumphosphid on rabbit eye, pharmatox, Hannover, Germany; unpublished report no. 1-3-184-87, 10/1987 (TOX2000-95)
Guidelines:	OECD guideline 405; Interpretation of the results according to the code of Draize (1959)
Deviations:	Neither the age nor the sex of the rabbits were reported. No information about the purity and the stability of aluminium phosphide, solid aluminium phosphide was instilled into the conjunctival sac of the rabbits and was washed out 30 seconds after application instead of 24 hours.
GLP:	Yes
Acceptability:	The study is considered to be supplementary.

Materials and methods:

Aluminium phosphide delivered by Degesch GmbH, Frankfurt/Main, Germany. Solid aluminium phosphide (0.1 g) was instilled into the conjunctival sac of 6 White New Zealand rabbits left eyes and was washed out 30 seconds after application. According to the guideline protocol the test substance should remain in the eye for 24 hours unless the substance is a known eye irritant. No reason is given for that. Since aluminium phosphide spontaneously hydrolyses if in contact with water (as it is the case in the eye) and since it is known that remaining aluminium hydroxide may cause irritations, it could have been reasonable to shorten the incubation period. The right eye stayed untreated but washed out and served as control. Readings of eye alterations were made after 1, 2, 4, 8, 24, 72 and 96 hours up to 7 days.

Findings:

Cornea and iris were unaffected by treatment. Slight conjunctival redness (grade 1) was recorded in all rabbits up to 8 hours and slight chemosis (grade 1) was recorded in 5/6 animals up to 1 hour after treatment. Therefore, aluminium phosphide is considered not to be irritating to the eye, if the test substance will be washed out 30 seconds after instillation into the conjunctival sac.

Conclusion:

Due to the short exposure time, no definitive statement about the eye irritating properties of aluminium phosphide can be derived from this study. However, the RMS proposes not to classify aluminium phosphide as an eye irritant, and is of the opinion that further testing is not needed based on the following considerations:

When the entry for aluminium phosphide in Annex 1 of Directive 67/548/EEC was last updated in 2001 (28th ATP or Dir. 2001/59/EC), no classification for eye irritation was assigned by the Commission Working Group on the Classification and Labelling of Dangerous Substances. It can be assumed that the present study, which was performed in 1987, has been part of the database available to the experts.

To the knowledge of the RMS, in decades of production and use of aluminium phosphide, no incidents of eye irritation in workers or applicators have been reported.

The Draize test for eye irritation causes great pain and suffering for the test animals, especially so when solids are applied. In addition, in the case of aluminium phosphide high systemic toxicity/mortality could be expected as a result of treatment with recommended dose levels. On the other hand, it remains doubtful, whether additional testing for eye irritation could significantly improve the overall risk assessment of aluminium phosphide.

In summary and in accordance with Dir. 67/548/EEC as last amended (29th ATP of 2004), it is proposed not to assign classification/labelling for eye irritation to aluminium phosphide.

Report:

Joshi, M. (1998): Mucous membrane irritation test of aluminium phosphide technical in rabbits, JAI Research Foundation (JRF), Gujarat, India, JRF study No. 365, date 15.10.1998 (TOX2006-217)

Guidelines:

Gaitonde subcommittee, Central Insecticide Board (CIB), India

Deviations:

Neither test substance purity nor batch number were mentioned.

Only very small amount of test material (1 mg) was tested. No information whether the applied substance was washed out.

Temperature of the experimental animal room was higher during the study (28-30 °C) instead of the recommended 20±3 °C.

Environmental conditions like air changes and photoperiod were not reported.

GLP:

No

Acceptability:

The study is considered to be not acceptable.

Materials and Methods:

In a primary eye irritation study, an amount of 20 mg of aluminium phosphide technical (purity not indicated) was instilled into an eye of one rabbit. This animal died within 3 h after instillation of the test substance. Due to the observed toxicity, only an amount of 1 mg of aluminium phosphide technical (purity not indicated) was instilled into the conjunctival sac of the left eye of six young adult New Zealand White rabbits (3 females and 3 males, supplied by Sai Biological Farm, Mumbai, India). No information was given whether the applied substance has been washed out. The contra lateral eyes served as controls. Animals were then

observed for 3 days. Both eyes of each animal were examined for signs of irritation at 24, 48 and 72 hours after dosing.

Findings:

Conjunctiva redness was noted in all animals after 24, an effect which had resolved by 24 hours (see Table B.6.2-20)

Table B.6.2-20: Eye irritation scores according to the Draize scheme

	Cornea						Iris						Conjunctiva											
													Redness						Chemosis					
Animal N°	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
24 h	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
48 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24-72 h mean	0						0						0.3						0					

Conclusion:

This study is insufficient to assess the eye irritating potential of aluminium phosphide, because only a small amount of aluminium phosphide technical was instilled into rabbit eyes and no information was given regarding the incubation period of the test substance.

Report:

Brunt, P. (2001): Zinc phosphide: Acute eye irritation in the rabbit; Safepharm Laboratories, Derby, U.K., Project No.: 1483/002, unpublished report, March 1, 2001 (TOX2005-171)

Guidelines:

OECD Guideline 405 'Acute Eye Irritation/Corrosion' (adopted 24 Feb 1987); Commission Directive 92/69/EEC Method B5

Deviations:

None

GLP:

Yes

Acceptability:

This study is considered to be acceptable.

Material and Methods:

Zinc phosphide, batch no.: 2000-10-24, purity: 82 %. Three (2 males and 1 female) New Zealand White rabbits (2.0 – 3.5 kg) were supplied by David Percival Ltd., Moston, Sandbach, Cheshire, UK. A volume of 0.1 mL/animal (246 µg) was placed into the conjunctival sac of the right eye. The left eye remained untreated as a control. Assessment of ocular damage/irritation was made approximately 1 hour and 24, 48 and 72 hours, and on day 7 following treatment. Evaluation was according to the classification schemes of Draize, Kay and Callandra.

Findings:

Slight initial pain reactions (blinking, trying to open eye, but reflex closing) were seen in all animals. Grey coloured staining of the fur was noted around the treated eye of two animals after 1, 24 and 48 hours. No evidence of corneal and iridial effects were noted during the study. Minimal to moderate conjunctival irritation was seen in all animals at the 1 hour observation and persisted throughout the 72 hours observation period. All treated eyes appeared normal on day 7. A maximum group mean score of 7.3 (1 and 24 hours) was calculated.

Conclusion:

The test material was classified as mild irritant according to the scheme of Kay and Callandra. However, no classification is required according to the classification criteria of the EU Commission Directive 93/21 EEC.

B.6.2.6 Skin sensitisation

No skin sensitisation study for calcium phosphide has been submitted. However, justification has been provided and accepted. The assessment of calcium phosphide is based on a study for zinc phosphide.

Report:	Brunt, P. (2001): Zinc phosphide: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation Method, SafePharm Laboratories, Derby, U.K.; unpublished report no. 1483/003, 25.04.2001 (TOX2002-179)
Guidelines:	OECD guideline 406, Commission Directive 96/54/EC method B6 Acute Toxicity (Skin Sensitisation)
Deviations:	This study does not concern the skin sensitisation of magnesium phosphide, but of zinc phosphide.
GLP:	Yes
Acceptability:	This study is considered to be acceptable.

Materials and methods:

Zinc phosphide, batch no.: 2000-10-24. The concentrations to be used in the main study were determined in preliminary tests. A group of 15 male albino guinea pigs was used for the main study, 10 test and 5 control.

Induction: Each test animal received intra dermal injections into the shaven shoulder. A row of 3 injections (0.1 mL each) was made on each side of the mid-line into a 20 mm x 40 mm area: Freund's Complete Adjuvant plus distilled water (1:1 ratio), a 0.5 % w/w formulation of the test material in distilled water, and a 0.5 % w/w formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water.

Approximately 24 and 48 hours after injection the degree of erythema at the test material injection site was evaluated. On day 7, the same area on the shoulder used previously was shaven again and a filter paper patch, loaded with the test material formulation (75 % w/w in distilled water) was applied to the skin, held in place with a strip of surgical adhesive tape and covered with aluminium foil. The degree of erythema and oedema was quantified 1 and 24 hours following removal of the patch after 48 hours. The same procedure was used for the control animals except that the test material was omitted from the injections and the topical induction.

Challenge: On day 21, both flanks of each animal were shaven. A filter paper patch loaded with an even layer of test material at maximum non-irritant concentration (50 % w/w in distilled water) was applied to the right flank. To ensure that the maximum non-irritant concentration was used, the test material at a concentration of 25 % w/w in distilled water was applied to the left flank. After 24 hours all patches were removed, and approximately after 24 and 48 hours the degree of erythema and oedema was quantified.

Findings:

50 % w/w in distilled water: No skin reactions were noted in the test or control group at both 24 and 48-hour observations.

25 % w/w in distilled water: Discrete or patchy erythema was noted in 2 test group animals at 24-hour observation, but were not apparent at the 48-hour observation. No skin reactions in the control group at both 24 and 48-hour observations.

Conclusion:

This study revealed no sensitising potential for zinc phosphide to guinea pig skin. Therefore, classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is not required.

B.6.3 Short-term toxicity (Annex IIA 5.3)

Calcium phosphide like other metal phosphides in contact with moisture readily decomposes to metal and phosphine, the toxicological principle. Due to the decomposition by moisture other phosphides are regarded as adequate model compounds. Studies with zinc phosphide, aluminium phosphide and phosphine are available.

In an oral 90-day gavage test (see Table B.6.3-1), mortality was increased at 2 mg aluminium phosphide/kg bw/d (corresponding to 1.18 mg PH₃/kg bw/d) in both sexes, the NOAEL being 1 mg aluminium phosphide/kg bw/d, equivalent to 0.59 mg PH₃/kg bw/d, respectively. However, these values are considered to be of limited reliability due to methodological deficiencies of the respective study report. A subchronic study in a second, non-rodent species was not submitted. An expert statement has been provided: The toxicological profile of calcium phosphide/PH₃ does not differ significantly between rodents and non-rodents and thereby justified non-submission of such data.

Male and female rats and mice were exposed up to 0, 1.25, 2.5 or 5 ppm PH₃ for 2 weeks. Under the conditions of this investigation the NOAEL was determined as 2.5 ppm PH₃ (0.95 mg/kg bw/day for rats, 0.1 mg/kg bw/day for mice) based on decreased lung weights in male rats/mice, increased heart weight in female rats/mice and increased urea nitrogen in mice at 5 ppm PH₃ (1.9 mg/kg bw/day for rats, 0.2 mg/kg bw/day for mice).

After inhalative administration of up to 3 ppm PH₃ gas (equivalent to ca. 1.1 mg/kg bw/d) to rats over a period of 90 days, no substance related adverse effects were observed. Two satellite groups at 5 and 10 ppm, respectively, were introduced during the course of the study. In the 5 ppm satellite group, which received the test item for only 2 weeks, no relevant effects were observed (which is in accordance with the NOAEL of 4.9 ppm in the inhalative developmental study in rats, see below). Inhalative administration of 10 ppm PH₃ (3.8 mg PH₃/kg/bw/d) was terminated after 3 days, when already 4/10 females had died. In summary, a short-term NOAEL of 1.1 mg PH₃/kg bw/d was established.

Table B.6.3-1: Overview on the short-term toxicity of metal phosphide and phosphine

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value NOAEL	Reference
Subchronic, oral, 13 week, Non-GLP	Rat, CFT- Wistar, 12F (female only)	Zinc phosphide 0, 50, 100, 200, 500 ppm	< 50 ppm (3.5 mg/kg bw/d)	Muktha Bai, K. et al. (1980), (TOX 2005-175)
Subchronic, oral, 90 d Non-GLP	Rat, Wistar 24M+24F 32M+32F (control)	Aluminium phosphide 0, 0.1, 0.5, 2 (week 1 and 2) 1 mg/kg bw	1 mg/kg bw (0.59 mg PH ₃ /kg bw)	Schnellhardt, M. et al. (1985), (TOX2005-282)
Subchronic, inhalation, 6h/day, 5d/week, 2 wks, Non-GLP	Rat, Fischer 344; Mouse, B6C3F1, 6M+6F	Phosphine gas (PH ₃) 0, 1.25, 2.5, 5 ppm	2.5 ppm = 0.95 mg/kg bw (rat) 0.1 mg/kg bw (mice)	Morgan, D.L. et al. (1995) (TOX2002-181)
Subchronic, inhalation, 6h/day, 5d/week, 2 – 4 wks, Non-GLP	Mouse, ICR, 10M	Phosphine gas (PH ₃) 5 ppm	No reliable NOAEL can be derived. Study not acceptable	Omae, K. et al. (1996) (TOX2002-174)
Subchronic, inhalation, 6h/day, 5d/week, 13 wks, satellite groups 3 resp. 13 days OECD 413; GLP	Rat, Fischer 344, 30M+30F, satellite 10M+10F and 6M+6F (control)	Phosphine gas (PH ₃) 0, 0.3, 1, 3, satellite groups: 5, 10 ppm	3 ppm = 1.1 mg/kg bw	Newton, P.E. (1990) (TOX2001-684)
Subchronic, inhalation, no guideline, no GLP	Rats (only male), cats and guinea pigs	Phosphine gas (PH ₃) 1, 2.5, 5 ppm No control groups!	No NOAEL can be derived. Study is not acceptable.	Klimmer, O.R. (1969), (TOX 96-52057)

1 ppm PH₃ is equivalent to 1.41 µg/L air, density of pure PH₃ (20 °C): (34 g/mol)/(24.1 L/mol) = 1.41 g/L
Assuming an hourly respiratory volume (rat) of 45 L/(h kg bw)

B.6.3.1 Oral subchronic toxicity

B.6.3.1.1 Oral subchronic toxicity, rat

Report:	Muktha Bai, K. et al. (1980): Short term toxicity study of zinc phosphide in albino rats (<i>Rattus norvegicus</i>), Ind. J. Exp. Biol. 18, 854-857, 1980 (TOX2005-175)
Guidelines:	No guideline study
Deviations:	Only female animals were used, parameters do not agree with guideline on subchronic toxicity.
GLP:	No
Acceptability:	The study is considered to be supplementary.

Material and methods:

Zinc phosphide (technical grade, purity 87 %) obtained from Swadeshi Chemicals (P) Ltd, Bombay was used. Individually caged female albino rats of CFT-Wistar strain weighing 58-71 g (28 day weanling) were statistically grouped. The rats were maintained in the animal house of the institute and were fed basal diet (cereal-pulses with 17 % protein) in uncooked form and tap water ad libitum. Zinc phosphide was mixed with the basal diet to give the required concentrations of 50, 100, 200 and 500 ppm. Groups of 12 rats were fed diet containing 0 (control), 50, 100, 200 and 500 ppm of zinc phosphide for 13 weeks. Daily food intake, weekly body weights, symptoms and mortality were recorded. At the end of experiment, rats were anaesthetised and blood samples at random for each dosage were examined for RBC, WBC, PCV, Hb and differential counts as described by Hepler (Manual of clinical laboratory methods, Thomas Springfield, USA, p. 33, 1950). Serum was separated and analysed for enzymes like SGOT, SGPT and alkaline phosphatase (ALP). SGPT (colorimetric assay using 2,4-dinitrophenyl hydrazine) and ALP (colorimetric assay using p-nitrophenyl phosphate method) and SGOT (colorimetric assay using 6-benzamido-4-methoxy-m-toluidine diazonium chloride) were estimated as described by Bergmeyer (Methods of enzymatic analysis, Vol. 2, Verlag Chemie Weinheim, pp. 556-760, 1974). Serum zinc content was analysed using Perkin-Elmer, Model 460 atomic absorption spectrophotometer by AOAC methods. Autopsies were conducted and macroscopic abnormalities were noted. Brain, thyroid, heart, liver, lungs, kidney, ovary, spleen and adrenals were weighed. Samples of these organs were preserved in 10 % formalin, paraffin-wax sections were stained with haematoxylin and eosin and subjected to histopathological examination. Urin collected during the last week of feeding was examined (both gross and microscopic) and tested for glucose, protein and bile pigments as described by Hepler.

Findings:

None of the rats succumbed to zinc phosphide at 50 and 100 ppm level. One rat died at 200 ppm level on the 7th day of feeding. However, among the 10 rats which succumbed at 500 ppm level, 7 died within 3 weeks, and the remaining 3 died during 9th, 11th and 13th week respectively. The rats showed symptoms like crouching, heavy breathing, ataxia and paralysis of hind limbs, while coma and convulsions occurred prior the death. Food intake decreased as the concentration of zinc phosphide increased in the diet. The cumulative ingestion of active ingredient by the rats which succumbed ranged from 118-1596 mg/kg bw while the maximum tolerated dose by the surviving was 317, 582, 1121 mg/kg bw for 50, 100 and 200 ppm respectively. Zinc phosphide at all dietary levels affected the body weight of growing rats significantly. The reduction in body weight was noticeable from 1st and 7th week of feeding when the level of zinc phosphide was 500 and 50 ppm, respectively. It was of interest to observe that in a few rats fed zinc phosphide (irrespective of the dosages) loss of hair was noticed which followed a regular pattern. Usually is started from the base of the tail and extended towards the head portion. This symptom generally appeared from the 6th week of feeding. At 50 and 100 ppm level although this symptom lasted only for few weeks, at higher concentrations this was more permanent and persistent. Rats also lost their body weight gradually succumbing to death. There were no significant differences in the weight of organs such as lungs, kidneys, spleen, adrenals and ovaries of rats fed zinc phosphide diet compared to control. Significant increase was noticed in the weights of brain, liver, heart and thyroids of rats fed 200 and 500 ppm. Histopathological examinations did not reveal any significant changes in various tissues of animals fed 50 and 100 ppm. At 200 ppm, slight pathological changes were observed in liver and kidney. However, at 500 ppm, bile duct proliferation and focal necrosis in liver, slightly atrophied glomeruli, few showing hyalinisation in kidney and necrosed cardiac tissue in the heart were discernible. The results of haematological tests in

treated rats were similar to those of controls except at 500 ppm, here low values were recorded for Hb, RBC, WBC and PCV. Increase in serum zinc content was noticed only in 200 and 500 ppm fed rats where the values were 33.3 ppm as compared to 16.6 ppm in controls. Further among the 3 enzymes analysed only alkaline phosphatase tended to show increased values (6.0- 8.2 U/L) in all the dosages fed (except 50 ppm) as compared to control (5.1 U/L). Urine analysis showed no significant results.

Conclusion:

The NOAEL of this study was < 50 ppm (equivalent to 3.5 mg/kg bw/d, on the basis of a cumulative dose of 317 mg/kg bw and 91 days of treatment) based on decreased body weight and loss of hair at 50 ppm.

Report:

Schnellhardt, M. et al. (1985): Untersuchungen zur subchronischen Toxizität von Aluminiumphosphid, 90-Tage-Test an wachsenden Wistarratten, Forschungszentrum für Tierproduktion, Dummerstorf-Rostock, Bereich Tierernährung „Oskar Keller“, Abteilung Blankenburg, DDR (TOX2005-282)
(Translation: “Study on the subchronical toxicity of aluminium phosphide, 90-day-test on growing wistar rats”)

Guidelines:

No

Deviations:

Test material technical AIP instead of Delicia Gastoxin, specification unclear, not mentioned whether dose level is based on pure AIP or Detia Gastoxin, stability not stated, no justification given for reduced dose level, no batch number mentioned.

GLP:

No

Acceptability:

The study is considered to be supplementary.

Materials and methods:

Test material was not Delicia-Gastoxin, instead, technical AIP was mixed 1 : 20 (w/w) with talcum to give a content of 35.2 mg AIP/g mixture, which was then mixed with the vehicle in appropriate proportions (no data given). It is not clear, whether dose levels (mg/kg bw/day) were based on pure AIP or Detia-Gastoxin (with AIP content of 56.7 %). No analytical verification of the stability or concentration of the test material has been provided, batch number has not been mentioned. Oral toxicity study was carried out over a period of 90 days on 208 rats (Bcp:WIST). Dose levels of 1 (2 mg/kg bw in the first and second week), 0.5 and 0.1 mg/kg bw were tested orally by gavage in 24 male and 24 female rats each, 32 male and 32 females in the control group. No justification was given for reduction of dose level from 2 down to 1 mg/kg bw/day after study week 2 in the highest dose group (but presumably due to high mortality). The compound was applied 5 days/week as a dilution in Systol T 122 (Polyethylene-glycol-Schwarzheide). Observations and examinations: Mortality, general appearance, behaviour, body weights, food consumption, haematology, clinical chemistry, urinalysis, organ weights, macroscopic and histopathological examinations were carried out with 8 male and 8 female rats in each dose-level (16 in the control).

Findings:

No particular differences on general condition and behaviour of animals were observed, except of three animals with otitis media (two in the 0.5 mg/kg dose-group and one in the control group) and some animals with dyspnoic symptoms in different groups. No significant difference persisted in body weights. Food consumption of the females in the 1 mg/kg group

was higher compared to the control group. Mortality was increased in the highest dose group (see Table B.6.3-2), but for the bulk of animals no clear cause of death could be established histopathologically. Insufficient data was provided, especially on the exact gavage process including applied volumes.

Table B.6.3-2: Mortality (%) in oral 90-day toxicity study in rats

Dose level (mg/kg bw/day)	Males	Females
0	12.5	15.6
0.1	0	0
0.5	12.5	16.7
2 (week 1 and 2) 1 (from week 3)	37.5	54.2

Only very slight effects on red blood as well as differential count with no/unclear toxicological relevance were observed at study termination. Changes in urea nitrogen, alkaline phosphatase and leucine amino peptidase were not dose related and seemed therefore not to be caused by the treatment with the test material. Treatment related decrease of the cholinesterase activity, which would indicate a reduction of the liver function, was not detected. Increased absolute and relative liver weights at all dose levels in males and at the intermediate dose in females were noted. This effect was assessed as an adaptive effect. Histopathological examinations revealed no changes in treated animals. In one animal of the 1 mg/kg bw/d dosage group, which died during the examination period a liver dystrophy, was detected. This could be treatment related, but to prove this fact only one incident is not sufficient.

Conclusion:

Under the conditions of this investigation the no observed adverse effect level (NOAEL) was determined as 1 mg/kg bw/day based on increased mortality at the LOAEL of 2 mg/kg bw/day.

B.6.3.1.2 Oral subchronic toxicity, dog

A short term toxicity study in dogs was not submitted. Justification for non-submission:

The submission or the conduct of such a study is not considered to be required for the following reasons:

(i) The toxic mechanism of magnesium phosphide via hydrolysis to the toxic phosphine gas is well known, involving inhibitory action on enzymes of electron transport mechanisms (IPCS, 1997¹) and also reaction with haeme proteins (Potter et al. 1991²). The mechanism of toxicity can therefore be considered not to be species-specific.

(ii) In view of the inorganic nature of the substance and the need for hydrolysis in the GI tract to elicit any toxicity, there is no reason to assume any relevant difference in uptake and metabolism between species.

¹ IPCS International Programme on Chemical Safety (1997): Poisons Information Monograph 865. Phosphine.

² Potter, W.T. et al. (1991): Phosphine-mediated Heinz body formation and haemoglobin oxidation in human erythrocytes. Toxicol. Lett. 57(1), 37-45.

(iii) Although only of indicative value, acute toxicity studies in rats, rabbits, guinea pigs, mice, cats and data in humans have yielded acute lethal concentration in a very narrow range, indicating that the species tested are similarly susceptible to phosphine (WHO, 1988³; IPCS, 1997⁵ Jokote, 1904⁴).

(iv) Similarly steep dose-response curves have been established across a range of species such as cats, rats, rabbits and guinea pigs after sub-acute or sub-chronic exposure (Klimmer, 1969⁵; Müller, 1940⁶, Newton, 1993⁷; Okolie et al., 2004⁸). In consideration of the arguments given above, there is no reason to assume that the dog is more susceptible than the rat to phosphine liberated upon ingestion of calcium phosphide. Thus, the generation of such data in a 90d-study in dogs is not likely to be of value for the extrapolation to man. As consequence, the conduct of such a study is not considered to be required, and should be avoided for animal welfare reasons.

B.6.3.2 Inhalation toxicity

B.6.3.2.1 14 – 28 day inhalation toxicity, rat and mouse

Report:	Morgan, D.L.; et al (1995): Inhalation toxicity of phosphine for Fischer 344 rats and B6C3F1 mice; Inhalation Toxicol. 7, 225 – 238 (TOX2002-181)
Guidelines:	No
Deviations:	Batch number and purity were not stated, several important data are missing (body weight gain, individual data, organ weights of pilot study, haematological parameter).
GLP:	No
Acceptability:	The study is considered to be supplementary.

Materials and Methods:

Phosphine gas (21500 ppm in nitrogen, AGA Speciality Gas Inc., Maumee, OH); batch no. and purity were not stated.

Test procedure: 4-day pilot study and subsequent 2-weeks whole-body inhalation toxicity study in B6C3F1 and Fischer 344 rats.

Pilot study: 5 males per group, additional 10 animals for the high dose group; exposure concentrations 0, 1, 5 and 10 ppm for 6 h/day.

Main study: 6 male and 6 female rats and mice per group; exposure concentrations: 0, 1.25, 2.5 and 5 ppm for 6 h/day, 5 days/week; Observations and examinations: mortality, body weights, phosphine determination in blood, lung, liver and kidneys, haematology, clinical chemistry, organ weights and histopathological examinations. Sacrifice: Immediately after the

³ WHO World Health Organisation (1988): Phosphine and selected metal phosphides, IPCS, Environmental Health Criteria 73, WHO, Geneva

⁴ Jokote, C.H. (1904): Experimentelle Studien über den Einfluß technisch und hygienisch wichtiger Gase und Dämpfe auf den Organismus, Teil XI. Studien über Phosphorwasserstoff. Arch. für Hyg. 49/50, 275-306.

⁵ Klimmer, O.R. (1969): Beitrag zur Wirkung des Phosphorwasserstoffes. Arch. Toxikol. 24 (2), 164-87.

⁶ Müller, W. (1940): Über Phosphorwasserstoffvergiftungen (Tierversuche). I. Mitt. Akute und subacute Vergiftung. Naunyn-Schmiedebergs Arch. Exp. Path. Pharmac. 239, 194-193.

⁷ IIA 5.2.3/03

⁸ Okolie, N.P. et al. (2004): Phostoxin-induced biochemical and pathomorphological changes in rabbits. Indian J Exp Biol. 42 (11), 1096-9.

4-day exposure period and immediately after the last exposure (males) or on the next morning (females) after the 2 week exposure period.

Findings:

Pilot study: Mean chamber concentrations were: 1.05 ± 0.06 , 4.98 ± 0.1 and 10.05 ± 0.44 ppm. No mice died during the 4-day study; however, all mice exposed to 10 ppm phosphine were in moribund condition after the last exposure. One rat died after 2 exposures to 10 ppm, 12 rats exposed to 10 ppm died during the third exposure and the remaining 2 rats died shortly after the third exposure. There were no significant treatment-related effects on haematological parameters in male rats exposed to 1 or 5 ppm. Statistically significant decreases in red blood cells, haemoglobin and haematocrit were observed in mice of the 1 and 10 ppm groups. Leucocytes were significantly decreased in the 10 ppm mice with concomitant decreases in lymphocytes and monocyte counts. Urea nitrogen, alanine-transferase and sorbitol dehydrogenase were significantly increased in mice of the 10 ppm group. Five of 6 moribund mice of the 10 ppm group which were sacrificed showed minimal to mild degeneration and necrosis of renal tubules, and minimal to mild myocardic degeneration and focal mineralisation of cardiac muscle fibres. Acid-labile phosphine was not detected in blood, kidneys, liver and lungs of mice exposed to 10 ppm. **Main study:** The mean chamber concentrations were: 1.19 ± 0.06 , 2.25 ± 0.69 and 5.14 ± 0.11 ppm. There were no mortalities in rats or mice in this study. After exposure of 2 weeks (see Table B.6.3-3), lung weights of male rats and male mice were significantly decreased, and heart weights of female rats and female mice were significantly increased at 5 ppm. Lung weights of male rats and male mice were significantly decreased after 3 days of exposure (data have not been submitted).

Table B.6.3-3: Organ weight (g) in rats and mice exposed to phosphine for 2 weeks

Dose level in ppm PH ₃	Male rats		Female rats	
	Lung	Heart	Lung	Heart
0	1.55 ± 0.16	0.70 ± 0.05	0.94 ± 0.12	0.52 ± 0.04
1.25	1.54 ± 0.26	0.74 ± 0.05	1.03 ± 0.183	0.54 ± 0.03
2.5	1.63 ± 0.21	0.76 ± 0.05	1.07 ± 0.21	0.54 ± 0.04
5.0	$1.23 \pm 0.15^*$	0.72 ± 0.06	0.96 ± 0.17	$0.65 \pm 0.06^*$
	Male mice		Female mice	
	Lung	Heart	Lung	Heart
0	0.27 ± 0.03	0.16 ± 0.02	0.22 ± 0.05	0.14 ± 0.01
1.25	0.27 ± 0.06	0.16 ± 0.04	0.21 ± 0.04	0.13 ± 0.01
2.5	0.30 ± 0.07	0.15 ± 0.02	0.24 ± 0.05	0.14 ± 0.01
5	$0.19 \pm 0.03^*$	0.16 ± 0.02	0.21 ± 0.05	$0.16 \pm 0.02^*$

*p < 0.05

With the exception of an increase of urea nitrogen in male mice exposed to 5 ppm, there were no significant treatment related changes in clinical chemistry parameters of rats and mice exposed to phosphine for 2 weeks. There was no microscopic evidence of treatment related effects in any of the tissues examined from rats and mice exposed to 5 ppm PH₃. Cardiomyopathy was seen in control (2/6 males, 1/6 females) and treated rats (1/6 males, 4/6 females), but with the exception of mild severity in one female, all other cases were of minimal severity. Tissues from animals of lower exposure groups were not examined. Acid-labile phosphine was not detected in blood, kidneys, liver and lungs of mice exposed to 5 ppm.

Conclusion:

Under the conditions of this investigation the no observed adverse effect level (NOAEL) was determined as 2.5 ppm PH₃ (0.95 mg/kg bw/day for rats, 0.1 mg/kg bw/day for mice) based on decreased lung weights in male rats/mice, increased heart weight in female rats/mice and increased urea nitrogen in mice at 5 ppm PH₃ (1.9 mg/kg bw/day for rats, 0.2 mg/kg bw/day for mice).

Report: Omae, K. et al. (1996): Acute and subacute inhalation toxicity of highly purified phosphine (PH₃) in male ICR mice; J. Occup. Health 38, 36 – 42 (TOX2002-174)

Guidelines: No

Deviations: Only one dose level, batch not stated; several important data are missing: analytical data from inhalation system, nutritional information, feed consumption and body weight, histopathological data.

GLP: No

Acceptability: The study is considered to be not acceptable.

Materials and Methods (subacute exposure):

Test material: Phosphine; batch no. not stated; purity: 99.995 %. Four week old male ICR mice (10 per group) were exposed to 5 ppm phosphine (PH₃) or filtered air (control) for six hours a day, five days a week, for two or four weeks in a whole-body inhalation experiment. This is an inadequate test design: only one dose group, not comparable to EU/OECD methodology, no dose response-relationship can be determined from this design. PH₃ was diluted with highly purified nitrogen used as the source gas, and was supplied at a constant flow rate mixed with room air and introduced into the 550 L exposure chamber. The exposure concentration was determined by gas chromatography. Mice were observed for behavioural changes, external appearance, mortality and body weight changes. One day after completion of exposure, blood was collected for haematological and clinical chemistry investigations, and animals were subjected to organ weight analysis and histopathological examinations. Several important data (a. o. analytical data from the inhalation system, nutritional information, feed consumption and body weight, histopathological data) are missing already in the original report.

Findings:

Exposure concentrations were 4.9 ± 0.3 ppm. Animals showed face washing and were extremely active after the start of exposure, but approximately one hour after start of exposure, their spontaneous motor activity diminished. Except for mild piloerection, there were no other particular findings. One animal of the 4 week exposure period died on day 12, and ventricular dilatation and pulmonary congestion were observed upon necropsy. However, in the absence of detailed histopathological data, and in the light of the fact that no mortality occurred in the 2 week exposure group, the relationship to treatment with phosphine of the one premature death in the 4 week group seems questionable. Body weight gains were significantly different from control after 4 weeks of exposure, the absolute weight of kidneys was significantly decreased. The organ weights of liver, spleen, thymus and kidneys are presented in Table B.6.3-4, whereas weights of lungs, testes, heart and brain that did not show statistically significant difference to control are not presented. However, the observed effects on organ weight were of a limited degree and it is difficult to judge on their relevance, as only data on absolute organ weights was provided.

Table B.6.3-4: Statistically significant effects on absolute organ weights (g)

	No. of animals	Liver	Kidneys	Spleen	Thymus
Control	9	1.33 + 0.05	0.47 + 0.03	0.10 + 0.02	0.06 + 0.01
2-weeks	10	1.22 + 0.10*	0.45 + 0.07	0.07 + 0.02*	0.05 + 0.01*
Control	10	1.30 + 0.13	0.56 + 0.04	0.08 + 0.02	0.03 + 0.01
4-weeks	9	1.29 + 0.08	0.52 + 0.04*	0.09 + 0.02	0.04 + 0.01

*p < 0.05

Histopathologic examinations revealed pulmonary congestion in one animal of the 4 week exposure group. Inflammatory changes in the mucosa of the nasal cavity were seen only in animals of the 4 week exposure group. Haematological investigations showed a significant decrease ($p < 0.05$) of monocytes after 2 weeks of exposure, and a significant increase ($p < 0.05$) of eosinophiles after 4 weeks. ALT and BUN were significantly increased ($p < 0.05$) in the 4 week exposure group. There were no differences in the mature sperm counts.

Conclusion:

Due to the lack of important data and an inadequate test design, this study is not suitable for risk assessment. No reliable NOAEL/LOAEL could be derived.

B.6.3.2.2 Inhalation 90-day toxicity – rat**Report:**

Newton, P.E. (1990): A thirteen week inhalation toxicity study of phosphine (PH_3) in the rat, Bio/dynamics, Inc., East Millstone, New Jersey, USA, unpublished report no. 87-8030, 23.02.90; exposure period: 07.09.1988 – 08.12.1988 (TOX2001-684)

Guidelines:

OECD Guideline: Repeat-Dose Inhalation Toxicity (May 1981), Part 413

Deviations:

Observations post exposure were carried out 5 days/week. No urinalysis. Lungs in animals from the low and intermediate dose groups were not histologically examined. Relative humidity during exposure ranged from 8 – 80%.

GLP:

Yes

Acceptability:

The study is considered to be acceptable.

Materials and methods:

Phosphine, 1.04 % average active ingredient in nitrogen; PH_3 was administered by whole-body inhalation as a gas to 240 Fischer 344 rats (30/sex/group). The test substance was administered for six hours per day, five days per week, for thirteen weeks at target concentrations of 0, 0.3, 1.0 and 3.0 ppm, groups I-IV, respectively. Ten animals/sex/group were sacrificed after 4 and 13 weeks of exposure and then after 28 days of recovery. In addition, satellite groups at 5 and 10 ppm (10 animals/sex: groups VIII and VI) and concurrent controls (6 animals/sex: groups VII and V) were exposed for 13 and 3 days, respectively, followed by a 28 day recovery period. Exposure levels were monitored by gas chromatography four times per chamber per day. Following the exposures, all animals were sacrificed, selected organs were weighed and organ/body weight ratios calculated. Complete gross post mortem examinations were conducted on all animals. Histopathologic evaluations were performed on selected tissues from all animals by Experimental Pathology Laboratories, Herndon, Virginia.

The cumulative mean analytical exposure concentrations, as determined by gas chromatography, were 0, 0.37, 1.0, 3.1, 5.1 and 10 ppm. The corresponding nominal concentrations for the phosphine exposed groups were 0.35, 0.99, 3.3, 5.1 and 9.1 ppm, respectively.

Findings:

Three 6-hour exposures to 10 ppm phosphine were fatal to female rats, resulting in the premature termination of this satellite group. No other deaths occurred. The borderline effects on body weight (gain), food consumption, and organ weights were mostly only slight and were not seen as being necessarily adverse. Besides, on most occasions there was no clear dose response relationship. Reduced testes weight was the only effect which was accompanied by a pathological correlate, i.e. small seminal vesicles with decreased secretion, but a marked effect was only seen at mid-dose. All other haematology and clinical chemistry effects seen at 5 ppm and lower exposure levels were completely reversible either during the exposure period or after a four week recovery period.

Conclusion:

Under the conditions of this investigation the no observed adverse effect level (NOAEL) was determined as 3 ppm PH₃. Assuming an hourly ventilation of 45 L/h/kg bw for the rat and a daily exposure time of 6 hours, this is equivalent to ca. 1.1 mg PH₃/kg bw/day.

B.6.3.2.3 Further inhalation studies

Report:	Klimmer, O.R. (1969): Contribution to the Study of the Action of Phosphine (PH ₃) - The Question of the So-Called Chronic Phosphine Poisoning, Arch. Toxikol. 24 (1969), pp. 164-187 (TOX96-52057)
Guidelines:	No
Deviations:	Parameter spectrum does not agree with guidelines; only male rats, low number of cats and guinea pigs, no control groups were used, different application frameworks in different dose groups.
GLP:	No
Acceptability:	The study is considered to be not acceptable.

Material and methods:

In the study a gas tight inhalation chamber having a capacity of 400 litres ('Würzburger Modell') was used. The chamber could be aerated. Phosphine/air mixture (Phosphonium iodide provided by Fa. Schuchardt, Munich) flowing continuously through the inhalation chamber 7 hours a day. Phosphine content was analysed before start of the test and every hour during inhalation tests ('flow test'). Phosphine was generated in an Erlenmeyer flask by dripping a 10 % KOH solution on pure phosphonium iodide. Phosphine dosage: 1 ppm, 2.5 ppm and 5 ppm respectively. At 1 ppm 4 female cats (2.1 to 2.95 kg) and 10 male wistar rats (110 g) were used. At 2.5 ppm 4 female cats (2.2 to 3.1 kg), 4 female guinea pigs (280 to 360 g) and 10 male wistar rats (110 g) were used. At 5 ppm in two subgroups a total of 6 white cats (2.2 to 3.3 kg), 6 female guinea pigs (300 to 360 g) and 20 male wistar rats (110 g) were used.

Test duration:

1 ppm: 5 weekdays of 6 hours each, Saturdays 4 hours, 24 weeks.
2.5 ppm: same, 24 weeks

5 ppm: 8 days per 6 hours each (sub group a) or 8 days per 6 hours each plus 4 days per 8 hours each (subgroup b); 48 and 80 hours respectively. Before starting the tests, at half-time and after the end of the experiments all the experimental animals were subjected to a urine analysis (protein, urobilinogen, sugar, sediment) and blood analysis (oxyhaemoglobin, methaemoglobin, osmotic resistance, blood status). Organs were histopathologically sectioned.

Findings:

1 ppm: experimental animals behaved calmly, mostly asleep and, with the exception of two cats having fallen sick intercurrently, exhibited no striking symptoms. The weights of the rats increased from an average of 110 g at the beginning to an average of 318 g at the end of the tests. In the urine of cats and rats no pathological material was found in addition to bacteria and crystals prior to the start of tests. Blood tests in cats revealed normal leucocyte count, blood colour, and the typing oxyhaemoglobin band, without any indication of methaemoglobin. Blood test on rats revealed normal osmotic impedance. The autopsy of the two cats and ten rats showed only a slight diffuse globular fatty infiltration in the liver parenchyma of the cats and a slight and isolated fatty infiltration in the adrenocortical system of the rats. Besides that, three rats exhibited a slight cloudy swelling of the tubular epithelia. The investigation of the brain section of all animals did not reveal any pathological findings.

2.5 ppm: The four female cats, four guinea pigs and ten rats behaved calmly, slightly increased in weight on the average, and survived with the exception of two rats which died after 742 and 708 test hours respectively of a proven pulmonary infection. The investigation of urine, blood counts and osmotic impedance of erythrocytes, showed a slight and uniform decrease of the erythrocyte and haemoglobin values for three out of four. Blood count, differential blood count and osmotic impedance were within the normal variation range. The three liver function tests made on each of the four cats showed normal bromsulphthalein excretion in all the cases. The blood showed only the oxyhaemoglobin band and not that of the methaemoglobin. Histopathological investigation revealed a slight diffuse fatty infiltration in livers of cats and an isolated slight cloudy swelling in the tubular epithelia of the rats. The neuropathological investigation of brain sections of cats, rats and guinea pigs revealed slight and non specific changes of the Purkinje cells, which, however, were considered as agonal or post-mortem changes.

5 ppm: All the animals showed a strong hyperaemia of the organs with cases of pulmonary oedema. The urine contained traces of albumen in 7 out of 10 cases. The blood of three cats, three guinea pigs and five rats showed only the oxyhaemoglobin band, a fact, however, which does not exclude the possibility of a slight methaemoglobin formation. The histological investigation of the organs of all the animals showed strong blood congestion in the capillaries, congestion in the liver with large vesicular-angular cells; a slight diffuse fatty infiltration was found in rats and guinea pigs, but not in cats. The neurohistological investigation of the brains showed for all the rats a striking dilatation of the perivascular areas, vacuolisation in the nuclei of the ganglion cells and decaying Purkinje cells, respectively.

Conclusion:

Subchronic exposure to 1 and 2.5 ppm phosphine did not result in any recognisable disturbance of blood formation, i.e. in shifts of haemoglobin values, erythrocyte and leucocyte counts, and in no disturbance of the osmotic impedance of the erythrocytes. The subacute phosphine poisoning at 5 ppm, however, was accompanied, in some animals, by a decrease of about 50 % in the haemoglobin and erythrocyte counts. Neither during the first three test series, however, nor during the ultimate acute tests on several animal species could the authors establish a noticeable formation of methaemoglobin.

B.6.4 Genotoxicity (Annex IIA 5.4)

All submitted in vitro bacterial reverse mutation tests (see Table B.6.4-1) showed negative results. No clear result was obtained for the potential of PH₃ to cause clastogenic effects in CHO cells in vitro. The results of the test were equivocal, however, the ability of the test design to detect potential clastogenic effects caused by PH₃ could not be demonstrated convincingly. 6 submitted in vivo tests (see Table B.6.4-2) showed negative results. In a subchronic (13 weeks, mice) in vivo test the formation of micronuclei was increased at the highest test concentration (approaching the LD₅₀). However, such exposure conditions are unlikely to be encountered in an occupational environment. In a dominant-lethal-test in mice with aluminium phosphide in peanut oil the post implantation loss was increased and the number of live implants was reduced. In the only dose level also toxic effects have been observed. However, the quality of the study was limited. An inhalative dominant-lethal test in mice was negative. Overall, calcium phosphide/PH₃ is not likely to be genotoxic in humans on relevant exposure conditions.

Table B.6.4-1: Summary of in vitro tests

Method	Test system (Organism, strain)	Concentra- tions tested	Results		Reference
			+ S9	- S9	
Bacterial reverse mutation test (Ames test)	Salmonella typhimurium, TA98, TA100, TA1535, TA1537, TA1538, Escherichia coli WP2 Hcr-	0-25600 ppm (estimate)	Negative	Negative	Sutou, S. et al. (1982) (TOX2005-283)
Bacterial reverse mutation test (Ames test)	Salmonella typhimurium, TA98, TA100, TA102, TA1535, TA1537, TA1538	0-4340 ppm	Negative	Negative	Stankowski, L.F. (1990) (2001-685)
Bacterial reverse mutation test (Ames test)	Salmonella typhimurium, TA98, TA100, TA102, TA1537, TA1535	0-1780 ppm	Negative	Negative	Rajwani, L.S. (2000) (TOX2006-220)
Bacterial reverse mutation test (Ames test)	Salmonella typhimurium, TA98, TA100, TA102, TA1537, TA1535, E. coli WP2uvrA	Phosphine gas up to 1 %	Negative	Negative	Araki et al. (1994) (TOX2002-182)
Structural chromosome aberration	CHO-K1-BH4 cells	0-4957 ppm	Equivocal	Equivocal	SanSebastian, J.R. (1990) (TOX2001-686)
Mammalian cell gene mutation (HGPRT test)	V79 hamster cells	0-6580 ppm	Negative	Negative	Leuschner, F. (1992) (TOX2005-284)

Table B.6.4-2: Summary of in vivo tests

Method	Species, Strain, Sex, No/sex/group	Route and Frequency of application	Sampling times	Dose levels	Results	Reference
Chromosomal aberration test in mice	Swiss albino mice	Single oral (gavage)	1 day post exposure	0-1.5-3-6 mg/kg bw	Negative	Guna Sherlin, D.M. (1998) (TOX2006-222)
Micronucleus test in mice	Swiss albino mice	2 days, oral (gavage)	1 day after last exposure	0-1.5-3-6 mg/kg bw	Negative	Guna Sherlin, D.M. (1998) (TOX2006-221)
UDS test in rat primary hepatocytes	Rat, CDF (F344)/CrIBR, M, 10	Single whole body inhalation, 6 h exposure time	At 2 and 12-14 h, respectively	0-4.8-13-18-23 ppm	Negative	McKeon, M.E. (1993) (TOX2005-285)
Test for micronuclei	Mouse, Balb-c, M, F, 4-6	Whole body inhalation, 2 weeks, 6 hours/day, 5 days/week	Not indicated	5.5+0.67 ppm	Negative	Barbosa, A. et al (1994) (TOX97-50676)
	M, F, 12	13 weeks, 6 hours/day, 5 days/week	Not indicated	0-0.3+0.1-1.0+0.2-4.5+0.8 ppm	Positive at the highest concentration	
Test for SCE, chromosome aberrations and micronuclei	Mouse, CD-1 (Charles River), M, 5	6 h inhalative exposure	At 20 hrs. post-exposure	0-5-10-15 ppm	Negative	Kligerman, A.D. et al. (1994) (TOX97-50677)
Test for SCE, chromosome aberrations and micronuclei	Mouse, CD-1 (Charles River), M, 3-5, Rat, F344/N (Charles River), M, 4-5	6 h/d inhalative exposure on 9 d during an 11 d period.	At 20 hrs. post-exposure	0-1.25-2.5-5 ppm	Negative	Kligerman, A.D. et al. (1994) (TOX2002-830)
Dominant lethal test	Mouse, B6C3F1 (Charles River), M, 50 (control: 30)	6 h/d inhalative exposure on 10 d during a 12 d period.	-	0-5 ppm		
Test for chromosome aberrations and micronuclei	Mouse (inbred swiss), 4	Zink phosphide, chromosome aberration test: acute: i.p., p.o. and s.c. Subacute: i.p., 5 days Micronucleus test: 2 x i.p. Sperm abnormality test: i.p., 5 days	24 h post exposure 6 h after last injection 35 days after first injection	20-20-40 mg/kg bw 8 mg/kg bw/d 20-30-40 mg/kg bw 20-30-40 mg/kg bw	Equivocal, however, study is not acceptable	Pal, B.B., Bhunya, S.P. (1995) (TOX2002-183)

Method	Species, Strain, Sex, No/sex/group	Route and Frequency of application	Sampling times	Dose levels	Results	Reference
Dominant lethal test	Mouse, Swiss albino, control: 10 M, treated group: 11 M	Aluminium phosphide in peanut oil	-	0-6 mg/kg bw/day	Positive at toxic concentration	Rajesh Sundar, S. (1999) (TOX2006-224)

B.6.4.1 In vitro genotoxicity testing – bacterial gene mutation assay

Report: Sutou, S. et al. (1982): In vitro microbial mutagenicity testing of hydrogen phosphide, Nomura Research Institute, Japan, unpublished report no. NRI 82-7492, 30.06.82 (TOX2005-283)

Guidelines: No guideline indicated.
The study was based on the following publications:
Ames, B.N. et al., Mutation Res., 31: 347 (1975)
Ames, B.N., Lee, F.D. & Durston, W.E., Proc. Natl. Acad. Sci. USA, 70: 782 (1973)

Deviations: Atmospheric levels of PH₃ are only estimates based on the amount of magnesium phosphide applied. No analytical verification, no analysis of residues.

Actual PH₃ levels within the test medium are subject to speculation.

Plates were exposed for only 1 hour.

In contrast, positive controls were applied as liquids.

GLP: No

Acceptability: The study is considered to be supplementary.

Materials and methods:

Hydrogen phosphide, generated by the reaction of magnesium phosphide and distilled water; batch no. 82016. The concentrations of hydrogen phosphide used were 640 ppm (ordinary concentration in actual usage), 1280 ppm, 2560 ppm, 6400 ppm, 12800 ppm and 25600 ppm.

Toxicity test of hydrogen phosphide with *S. typhimurium* TA98:

S. typhimurium TA98 cultured overnight was diluted to 10⁻⁶ with 0.1 M phosphate buffer. One-tenth mL of the diluted culture was plated on Vogel-Bonner E. agar medium and the bacteria were exposed to hydrogen phosphide for one hour at the ambient temperature. After incubation for two days at 37 °C, the number of colonies was counted. Three plates were used for each prescribed concentration. Reverse Mutation-Plate Method with *E. coli* B/r WP2 TRP-HCR:

Tryptophan-requiring *E. coli* WP2 Her- was cultured overnight with nutrient broth. Ten mL of 0.5 mM tryptophan solution was added to 100 mL of the top agar, which consisted of 0.5 % NaCl and 0.7 % DIFCO agar. Then, 0.1 mL of overnight culture of *E. coli*, 2 mL of the molten tryptophan-containing top agar, and 0.5 mL of 0.1 M phosphate buffer or 0.5 mL of S9 mix were mixed in a small test tube and poured onto Vogel-Bonner E. agar medium. Duplicate plates were placed in the exposing apparatus and exposed to hydrogen phosphide for one hour at the ambient temperature. After incubation for three days at 37 °C, the numbers of revertant colonies were counted. Dimethyl sulfoxide was used as the negative control, and 2-aminoanthracene (2AA) in the presence of S9 mix and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) in the absence of S9 mix were used as the positive control. The mean number of revertant colonies was calculated for each dose level.

Reverse Mutation-Plate Method with *S. typhimurium*:

Histidine and biotin-requiring *S. typhimurium* TA 1535, TA 100, TA 1537, TA 1538 and TA 98, which were established by Ames and others, were used. Each strain of *S. typhimurium* was cultured overnight with nutrient broth. A mixture (10 mL) of 0.5 mM histidine and 0.5 mM biotin was added to 100 mL of the top agar, which consisted of 0.5 % NaCl and 0.7 % DIFCO agar. Then, 0.1 mL of overnight culture of *S. typhimurium*, 2 mL of the molten histidine and biotin-containing top agar, and 0.5 mL of 0.1 M phosphate buffer or 0.5 mL of S-9 mix were mixed in a small test tube and poured onto Vogel-Bonner E. agar medium. Duplicate plates were placed in the exposing apparatus and exposed to hydrogen phosphide for one hour at the ambient temperature. After incubation for three days at 37°C, the numbers of revertant colonies were counted. As the negative control, dimethyl sulfoxide was used. The positive controls were N-ethyl-N'-nitro-N-Nitrosoguanidine (ENNG) in the absence of S9 mix and 2AA in the presence of S9 mix for TA 1535, AF-2 in the absence of S-9 mix and 2AA in the presence of S-9 mix for TA100 and TA98, 9-amininoacridine (9AC) in the absence of S-9 mix and 2AA in the presence of S-9 mix for TA 1537, and 2-nitrofluorene (2NF) in the absence S-9 mix and 2AA in the presence of S-9 mix for TA 1538.

Findings:

In the toxicity test of hydrogen phosphide with *Salmonella typhimurium* TA 98, approximately 20 % growth inhibition was observed at the highest concentration, but no toxic effect was observed at lower concentrations. Reverse mutation with *Escherichia coli* WP2 and *S. typhimurium* TA 1535, TA 100, TA 1537, TA 1538 and TA98 in the presence and absence of S9 mix, a metabolic enzyme activation system, was conducted. No mutagenic activity of hydrogen phosphide was detected.

Conclusion:

No mutagenic activity of hydrogen phosphide was detected regardless of the absence or presence of S9 mix. From the above test results, it can be concluded that hydrogen phosphide was negative in this gene mutation assay.

Report:

Stankowski, L. F. (1990): Ames/Salmonella Plate Incorporation Assay on Hydrogen Phosphide (PH₃), Pharmakon Research International, Inc., Waverly, Pennsylvania, USA, unpublished report no. PH 301-DA-001-89, 10.02.90 (TOX2001-685)

Guidelines:

No guideline indicated.

The study was based on the following publications:

Ames, B.N. et al (1975): Methods for detecting carcinogens and mutagens with the Salmonella/Microsome Mutagenicity, Mutation Res., 31: 347 – 364

Maron, D.M. and B.N. Ames (1983): Revised methods for the Salmonella mutagenicity test, Mutation Res. 113: 173 – 215

Maron, D.M. et al. (1981): Compatibility of organic solvents with the Salmonella/Microsome Test, Mutation Res., 88: 343 – 350

Snee, R.D. and Irr, J.D. (1981): Design of a statistical method for the analysis of mutagenesis at the hypoxanthine guanine phosphoribosyl transferase locus of cultured Chinese hamster ovary cells, Mutation Res., 85: 77 – 93

- Deviations:** Some of the positive controls (1,3-butadiene, ethylene oxide) were successfully employed as gases, demonstrating in principle, that uptake of compounds from the gas phase by the target cell cultures was possible in the test system. However, uptake of a gaseous substance by cultured bacterial cells might heavily depend on its physico-chemical properties and with phosphine the situation might be completely different from that of the above compounds.
- GLP:** Yes
- Acceptability:** The study is considered to be acceptable.

Materials and methods:

Hydrogen phosphide, 10000 ppm in N₂ as a clear colorless gas, was evaluated in the Ames/Salmonella Plate Incorporation Assay to determine its ability to induce reverse mutations at selected histidine loci in six tester strains of Salmonella typhimurium in the presence and absence of an exogenous metabolic activation system (S9). Based upon an expected lack of toxicity, PH₃ was evaluated in triplicate cultures in strains TA 1535, TA 1537, TA 1538, TA98, TA100 and TA102 in the presence and absence of S9 at doses of 4.52, 54.7, 190, 488, 1160 and 4340 ppm (all doses are based upon analytically determined concentrations from analysis performed by the sponsor). Due to the findings reported below, PH₃ was re-evaluated in the confirmatory assay in all six strains at doses of 73.3, 147, 228, 360, 378 and 399 ppm with and/or without S9. PH₃ was re-evaluated in a third assay in all six strains at doses of 99.0, 111, 152, 172, 183 and 262 ppm with and without S9.

Findings:

In the Ames/Salmonella Plate Incorporation Assay inhibited growth was observed in all tester strains at doses > 488 ppm with and without S9. Revertant frequencies for all doses of PH₃ in all strains with S9, and in strains TA1537, TA 1538, TA98, TA100 and TA102 without S9, approximated or were less than those observed in the concurrent negative control cultures. In contrast, a statistically significant increase in revertant frequency, to approximately 2.5 fold control values, was observed in strain TA1535 at a dose of 190 ppm without S9. In addition, the increase was apparently dose dependent over the range of 0 – 190 ppm. PH₃ was re-evaluated in the confirmatory assay in all six strains at doses of 73.3, 147, 228, 360, 378 and 399 ppm with and/or without S9. Inhibited growth was again observed in all tested strains at doses of 146.5, 228, 360, 378 and/or 399 ppm with and without S9. Revertant frequencies for all doses of PH₃ in strains TA1535, TA1538, TA100 and TA102 with S9, and all strains without S9, approximated or were less than control values. Increased revertant frequencies, to approximately 3.2- to 4.5 fold control values, were observed in strains TA1537 and TA98 at a dose of 360 ppm with S9. However, these increase were neither statistically significant nor dose dependent (revertant frequencies at doses of 228, 378 and 399 ppm were below control values). PH₃ was re-evaluated in a third assay in all six strains at doses of 99.0, 111, 152, 172, 183 and 262 ppm with and without S9. Normal growth was observed in all strains at all doses with and without S9. Revertant frequencies for all doses of PH₃ in strains TA1535, TA1537, TA98, TA100 and TA102 with S9, and all strains without S9, approximated or were less than control values. In contrast, statistically significant increases in revertant frequencies, to approximately 2.1 fold control values, were observed in strain TA1538 with S9. Although these increases were apparently dose dependent, revertant frequencies at all dose levels were elevated as compared to control values. PH₃ was subsequently re-evaluated in all six strains with and without S9 in two additional assays. Revertant frequencies for all doses of PH₃ in all six strains in the fourth assay approximated or were less than control values at doses of 37.0 –

203 ppm with and without S9 (although no toxicity was apparent). Similar results were observed in the fifth assay at doses of 41.0, 139, 277, 518, 670 and 962 ppm (however, significant toxicity was observed in this last assay at doses of 277, 518, 670 and/or 962 ppm with and/or without S9). All positive and negative control values in all assays were within acceptable limits.

Conclusion:

Thus, the increases observed in *S. typhimurium* strains TA1535, TA1537, TA1538 or TA98 in the first three assays were never independently confirmed and are considered to be statistical aberrations or artifactual in nature. Therefore, the results for PH₃ were negative in the Ames/Salmonella Plate Incorporation Assay.

Report:	Rajwani, L.S. (2000): Salmonella typhimurium reverse mutation assay of aluminium phosphide technical. JAI Research Foundation (JRF), Gujarat, India, JRF study No. 2567, 14.09.2000 (TOX2006-220)
Guidelines:	Environmental Protection Agency (1998) Health effects test guidelines. OPPTS 870.5100
Deviations:	Cytotoxicity was not demonstrated. No positive control substances were tested. No repeat assays for mutagenicity were performed.
GLP:	Yes (laboratory certified by Netherlands authorities).
Acceptability:	The study is considered to be acceptable.

Materials and Methods:

Aluminium phosphide techn., description: yellowish green coloured powder, lot/batch #: 9/99, purity: 85.65 %, stability of test compound: stable in aluminium flask, solvent used: none; phosphine gas was generated from aluminium phosphide with a special gas generation assembly connected to an exposure.

S9 mix composition (10 mL):

Component:	Concentration:
Distilled water	3.8 mL
Phosphate puffer	5.0 mL
MgCl ₂ -KCl salt solution	0.2 mL
Glucose- 6-phosphate (1M)	0.1 mL
NADP (0.1 M)	0.4 mL
S9 fraction	0.5 mL

Test organism: *Salmonella typhimurium* strains: TA 1535, TA 1537, TA 98, TA 100 and TA 102, test organisms were properly maintained and were checked for appropriate genetic markers (rfa and uvrB mutation, R factor).

Test concentrations: Preliminary cytotoxicity assay:

strain TA 98	0, 280, 670, 860, 1260 and 1628 ppm
strain TA 100	0, 250, 592, 914, 1185 and 1582 ppm
strain TA 102	0, 243, 575, 950, 1130 and 1640 ppm
strain TA 1537	0, 310, 652, 850, 1290 and 1780 ppm
strain TA 1535	0, 290, 638, 870, 1330 and 1750 ppm

Mutation assay:

strain TA 98	0, 188, 710, 1004, 1380 and 1710 ppm
strain TA 100	0, 145, 720, 984, 1565 and 1625 ppm
strain TA 102	0, 185, 812, 1015, 1440 and 1714 ppm
strain TA 1537	0, 185, 630, 871, 1420 and 1625 ppm
strain TA 1535	0, 190, 734, 905, 1275 and 1590 ppm

Tests were carried out from 08.08.2000 to 14.08.2000

Plate incorporation assay (for preliminary cytotoxicity and mutation assays):

Metabolic activation: Post mitochondrial supernatant (S9 fraction) were received from DRDE, Gwalior, India. Before use S9 fraction was supplemented with co-factors. Procedure: Tests were run in absence of metabolic activation and with a metabolic activation system (S9 mix). In the cytotoxicity test five concentrations of aluminium phosphide technical were tested for all five strains using two plates per concentration. The mutagenicity tests were run with all five strains and three plates per concentration as well as negative controls. Mixtures of bacterial cultures, top agar, activation mixture (for experiments in presence of S9) or phosphate puffer (for experiments without S9) were poured on agar plates. After solidification of the agar mixture the plates were exposed for two hours to different concentrations of aluminium phosphide. After incubation at 37 ± 1 °C for 48 to 72 hours and examine of background bacterial growth, cytotoxicity was assessed.

Pre-incubation assay: Prior to the mutagenicity test, aluminium phosphide technical was assessed for cytogenicity to bacteria. Test strains were tested against TA1537, TA1535, TA98, TA100 and TA102 with and without 5 % (v/v) S9 mix. Five different concentrations of aluminium phosphide in the range of 243 and 1780 ppm were tested for each of the five tester strains. After exposure of the Petri dishes, that contained the bacteria, and incubation at 37 ± 1 °C for 48 to 72 hours the state of background bacterial growth was assessed.

Statistics: Linear regression analysis.

Evaluation criteria: The test material is considered positive for a particular strain and condition if it caused a statistically significant ($p \leq 0.01$) dose-related increase in revertants over the solvent controls at three treatment levels.

Findings:

Five concentrations of phosphine gas ranging from 243 to 1780 ppm were evaluated \pm S9 in five strains. No cytotoxicity was observed. Details are shown in Table B.6.4-3.

Table B.6.4-3: Cytotoxic effects of aluminium phosphide

Aluminium phosphide [µg/plate]	S9	TA 1537		TA 1535		TA 98		TA 100		TA 102	
		-	+	-	+	-	+	-	+	-	+
Control (S9 mix)		NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
243 - 310 ppm		NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
575 - 670 ppm		NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
850 - 950 ppm		NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
1130 - 1330 ppm		NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
1582 - 1780 ppm		NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

NI no inhibition

Mutation assay: Five concentrations of phosphine gas ranging from 145 to 1714 ppm were evaluated \pm S9 in five strains. No cytotoxicity was observed. Revertant counts in phosphine treated tests did not differ significantly from the negative (culture medium) control data. Details are shown in Table B.6.4-4.

Table B.6.4-4: Mean number of His⁺ revertant colonies per plate

Strains		TA 1537		TA 1535		TA 98		TA 100		TA 102	
Aluminium phosphide [µg/plate]	S9	-	+	-	+	-	+	-	+	-	+
Control (S9 mix)		9.00	8.67	11.00	14.67	20.67	53.00	110.33	128.33	293.00	300.00
145 – 190 ppm		8.33	13.00	17.67	13.67	23.00	45.00	121.00	130.67	304.33	325.00
630 – 812 ppm		10.00	9.33	16.33	14.33	17.67	37.33	116.00	112.00	282.33	326.67
871 – 1015 ppm		8.33	10.00	12.67	16.00	25.33	24.00	96.67	109.67	297.67	327.67
1275 – 1565 ppm		8.67	10.33	14.67	16.00	29.33	45.67	130.00	122.33	288.67	328.67
1590 – 1714 ppm		9.33	8.67	17.00	16.00	37.00	47.67	117.33	115.00	298.33	327.00

Conclusion:

It was concluded that phosphine gas generated from the test article was not mutagenic in this bacterial test system, either in the presence or absence of metabolic activation in the strains tested. The sensitivity of the procedures to detect mutagenesis was not validated by the inclusion of positive controls.

Report: Araki, A.; et al. (1994): Improved method for mutagenicity testing of gaseous compounds by using a gas sampling bag, published in: Mutat. Res. 307, pp. 335-344, 1994 (TOX2002-182)

Guidelines: No guideline indicated.

Deviations: No batch and purity indicated.

GLP: No

Acceptability: The study is considered to be supplementary.

Material and methods:

Phosphine (Nihon Sanso Co. Ltd., Tokyo, Japan), batch no. and purity: no data. Test procedure: reverse mutation assay with and without metabolic activation using Escherichia coli WP2 uvrA and four strains of Salmonella typhimurium (strains: TA98, TA100, TA1535, TA1537), Metabolic activation: S9-mix from male Sprague Dawley rats, Method: bacterial plates prepared by the agar overlay method were placed in 10-l gas sampling bags. The air in the bag was removed and replaced by the test substance gas (phosphine diluted with helium) at an adjusted concentration level. Exposure volume was 500 mL/plate, and maximum concentration of phosphine was limited to 1 %. After 2 hours of exposure at 25 °C, the test substance gas was removed, and sterile air was pumped into the exposure bag for 30 min. Thereafter, plates were incubated for 48 hours at 37 °C.

Findings and Conclusion:

Phosphine was not mutagenic to S. typhimurium TA98, TA100, TA1535 and TA1537 and E. coli WP2 uvrA with and without metabolic activation under the conditions of this test.

B.6.4.2 In vitro genotoxicity testing – clastogenicity test in mammalian cells

Report: SanSebastian, J.R. (1990): Structural Chromosome Aberration Chinese Hamster Ovary (CHO) Cell induced by Hydrogen Phosphide (PH₃), Pharmakon Research International, Inc., Waverly, Pennsylvania, USA, unpublished report no. PH 320-DA-001-89, 08.03.90 (TOX2001-686)

Guidelines: No guideline indicated.
According to the authors the study was based on the following publications:
Buckton, K.E. and Evans, H.J. (1973): Methods for the Analysis of Human Chromosome Aberrations. WHO, Geneva
Goto, K., Maeda, S., Kano, Y. and Sugiyama, T. (1978): Factors involved in differential Giemsa – staining of sister chromatids. Chromosoma 66: 351 – 359
EPA New and Revised Health Effects Guidelines, Office of Pesticides and Toxic Substances, Report No. EPA 560/6-82-001, 1983. Environmental Protection Agency Federal Register Vol. 50, No. 188, Friday, September 27, 1985
Perry P., and Wolff S. (1974): New Giemsa method for the differential staining of sister chromatids. Nature 251: 156 – 158
Preston, J. et al. (1981) : In Vivo and In Vitro Cytogenetic Assays : A Report of the U.S. EPA « Gene-Tox » Program. Mutation Research 87: 143 – 188
Savage, J.R.K. (1975): Classification and Relationships of Induced Chromosomal Structural Changes. Journal of Med. Genetics 12: 103 – 122

Deviations: No assessment of the levels of BD or PH₃ in the culture medium was performed. No relevant cytotoxicity was observed at any dose level. This could mean that PH₃ levels within the culture medium were too low to cause cytotoxicity. This might be seen as indicative of the test system also not being sensitive enough to detect a potential clastogenic effect of PH₃.
Mean cell cycle and average proliferation time used to identify cytotoxicity
150 metaphases scored per concentration

GLP: Yes

Acceptability: The study was considered to be acceptable.

Materials and methods:

Hydrogen phosphide, 10000 ppm in N₂ as a clear colourless gas. In a preliminary cytotoxicity test utilising cell proliferation kinetics as a parameter in CHO cells the appropriate concentrations for the assay were determined as 500, 2500 and 5000 ppm.

In the chromosome aberration assay in CHO cells, duplicated roller bottles were inoculated with 1 x 10⁶ CHO-K1-BH4 cells/bottle (Lot #A-12 and A-1 from Dr. Abraham W. Hsie, Tennessee, USA). Each dose was evaluated either with or without S-9 mix along with current untreated, solvent and positive controls. Cell cultures were treated for five hours in culture medium containing PH₃ or the control articles followed by three Saline-G washes and medium replacement. The cultures were then incubated for an additional 8, 18 and/or 26 hours. Two to

three hours prior to harvest, colcemid was added to arrest cells in mitosis. Cultures were harvested at the appropriate time and slides were prepared, stained and coded. 150 metaphases from duplicate cultures were pooled for analysis.

Findings:

MNNG at 15 µg/mL produced statistically significant increases in aberration frequency and proportion of aberrant metaphases. The positive response of MNNG demonstrates the integrity of the assay. However, BD at 50 % did not produce any statistically significant increase at 18-hour harvest time. The positive response of BD at the 8-hour time interval, however, is probably due to the low mean of the solvent control, since the mean of BD is within the historical range for the solvent control. This lack of a true positive response for BD indicates that the S9 activation system was not functioning biologically or perhaps, the BD was not tested at the appropriate dose to induce structural chromosomal aberrations. Results indicate PH₃ did not produce any statistical significance at the 18 and 26-hour time intervals with and without S9 mix. However, PH₃ induced statistically significant increase in aberrations per cell at 2500 (2733) and 5000 (4957) ppm without S9 mix at the 8 hour time interval.

Conclusion:

In conclusion, PH₃ did not produce any statistical significance at the 18 and 26-hour time intervals with and without S9 mix. However, PH₃ induced statistically significant increase in aberrations per cell at 2500 (2733) and 5000 (4957) ppm without S9 mix at the 8 hour time interval. To sum up it can be said, that no clear results were obtained for the potential of PH₃ to cause clastogenic effects in CHO cells *in vitro*. The ability of the test design to detect potential clastogenic effects caused by PH₃ could not be demonstrated convincingly.

B.6.4.3 In vitro genotoxicity testing – gene mutation assay in mammalian cells

Report:	Leuschner, F. (1992): Phosphine mutagenicity study in mammalian cells (V79) in vitro – HGPRT-Test, LPT, Hamburg, Germany, unpublished report no. 6990/91, 15.06.92 (TOX2005-284)
Guidelines:	OECD Guideline 476
Deviations:	Positive controls were apparently applied as liquids/solutions. They can therefore not be taken as reliable indicators for the suitability of the test system. No assessment of the levels of PH ₃ in the culture medium was performed.
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Phosphine, batch no. LS 109 25, gaseous, 0.658 % phosphine (v/v), balance nitrogen. Test samples of phosphine were assayed in a gene mutation assay in cultured mammalian cells (V79, genetic marker HGPRT) both in the presence and absence of metabolic activation by a rat liver post-mitochondrial fraction (S9 mix) from Aroclor 1245-induced animals. The duration of the exposure with the test substance was 24 hours in the experiments without S9 mix and 2 hours in the experiments with S9 mix. The exposure concentrations in the air for the study ranged from 500 to 6580 ppm (0.57 – 9.30 mg/L air) phosphine for the studies

without and with metabolic activation. For technical reasons it is not possible to employ phosphine concentration above 6580 ppm in the presence of air as phosphine may otherwise auto-ignite. Ethyl methanesulfonate (EMS) was employed as positive control chemical in the absence of metabolic activation and 9,10-dimethyl-1,2-benzanthracene (DBMA) was employed as positive control chemical in the presence of metabolic activation.

Findings:

At none of the doses up to and including 4000 ppm cytotoxicity or an increased mutation rate were observed, therefore it is not possible to judge whether the target cells within the culture medium were really exposed to PH_3 at all. On the other hand, at the highest dose level of 6580 ppm, cytotoxicity was likely to have been caused by the lack of oxygen/carbon dioxide, as no air was admixed to the test substance (0.658 % v/v PH_3 in nitrogen). Under the present test conditions phosphine tested up to an exposure concentration of 6580 ppm in the air in the absence and presence of metabolic activation in two independent experiments was negative in the V79 mammalian HGPRT cell mutagenicity test under conditions where the positive controls exerted potent mutagenic effects.

Conclusion:

No conclusions regarding the potential of PH_3 to cause mutagenic effects in V79 hamster cells can be drawn from this test. The ability of the test design to detect potential mutagenic effects caused by PH_3 could not be demonstrated convincingly.

B.6.4.4 In vivo genotoxicity testing

Report:	Guna Sherlin, D.M. (1998): Chromosomal aberration test of aluminium phosphide technical in mice. JAI Research Foundation (JRF), Gujarat, India, JRF study No. 367, 23.11.1998 (TOX2006-222)
Guidelines:	“The recommendations on pesticide toxicology submitted to Central Insecticide Board by Dr. B.B. Gaitonde, protocols for mutagenicity tests, micronucleus test”
Deviations:	No range finding toxicity test was performed. Test substance purity not indicated.
GLP:	None
Acceptability:	The study is considered to be supplementary.

Material and methods:

Aluminium phosphide techn., description: grayish colour powder, lot/batch: not reported, purity: not reported, stability of test compound: stable in aluminium bottle at room temperature, solvent used: peanut oil, controls: positive: intraperitoneal dose of 4 mg Mitomycin-C/kg bw, solved in distilled water, dose volume of 10 mL/kg bw, test animals: mice, strain: Swiss albino, age: 6 - 7 weeks, weight at dosing: 22 - 31 g source: breeding facilities at JAI Research Foundation, India, acclimation period: 5 days diet: mice pellet diet (Amrut brand), Nav Maharashtra Chakan Oil Mills Ltd, India, ad libitum water: drinking water (filtered through Aquaguard water filter system), ad libitum housing: animals were housed 4 per cage in polypropylene cages with stainless steel grid top, with rice husk as bedding, number of animals per dose: 4 animals/group, test compound concentrations: 0, 1.5, 3.0 and 6.0 mg /kg bw, dose volume: 10 mL/kg bw, tests were carried out from 10.10. to 23.11.1998. Animals received the test compound by a single oral application (gavage). Control group

received only peanut oil, whereas mice from positive control received a single application of Mitomycin-C dissolved (4 mg/kg bw) in distilled water. The application volume was 10 mL/kg bw. On the day after treatment, animals received 3 hours prior sacrifice an intraperitoneal injection of aqueous solution of colchicines at dose level of 4 mg/kg bw to arrest cell divisions at metaphase. For bone marrow preparation animals were sacrificed by cervical dislocation. Femora were excised and epicondyle tips removed. Bone marrow was harvested. Smears prepared from the bone marrow serum was place on a slide and stained with 5 % Giemsa in phosphate buffer. Slides were examined for chromosomal aberration, among 500 cells per animal were counted to determine the mitotic index. Among 100 metaphases per animal were observed for structural and numerical anomalies and pulverisation were recorded. Statistical methods: Student's t-test.

Findings:

No deaths occurred. Clinical signs (tremor, polyurea, and piloerection) were observed after dosing at 6 mg/kg bw. There were no significant decreases in mean body weight change for any of the treated groups or control groups (vehicle and positive control). There was no relevant difference in the incidence of aberrant cells between treated and vehicle control groups (see Table B.6.4-5). The incidence of aberrant cells in the positive control group fulfilled the criteria of a positive response and demonstrated the sensitivity of the test system.

Table B.6.4-5: Summary of results of a chromosomal aberration assay in male mice

Parameter	Vehicle control	1.5 mg/kg bw	3.0 mg/kg bw	6.0 mg/kg bw	Positive control #
Mitotic index	2.33 ± 0.38	2.80 ± 0.50	2.69 ± 0.17	2.66 ± 0.80	1.93 ± 1.03
Aberrant cells (%)	0.75 ± 0.50	0.50 ± 0.58	0.50 ± 0.58	1.00 ± 1.41	12.0 ± 2.94*

vehicle control: peanut oil (10 mL/kg bw per os); positive control: mitomycin-C (4 mg/kg bw i.p.)

* significant at 5 % level ($p \leq 0.05$)

Conclusion:

Aluminium phosphide technical does not induce chromosomal aberrations in mice up to a dose of 6 mg/kg bw by oral gavage (one-half of LD₅₀).

Report:

Guna Sherlin, D.M. (1998): Micronucleus test of aluminium phosphide technical in mice. JAI Research Foundation (JRF), Gujarat, India, JRF study No. 368, 23.11.1998 (TOX2006-221)

Guidelines:

"The recommendations on pesticide toxicology submitted to Central Insecticide Board by Dr. B.B. Gaitonde, protocols for mutagenicity tests, micronucleus test"

Deviations:

No range-finding toxicity test was performed. Bone marrow was sampled 24 hours after dosing only. No historical control data on micronucleated polychromatic erythrocytes were presented. Test substance purity and batch number were not indicated.

GLP:

No

Acceptability:

The study is considered to be supplementary.

Material and Methods:

Test Material: aluminium phosphide techn., description: grayish coloured powder, lot/batch: not reported, purity: not reported, stability of test compound: stable in aluminium bottle at room temperature, solvent used: peanut oil, control materials: positive: Mitomycin-C solved in distilled water test animals - species: mice, strain: Swiss albino, age: 6-7 weeks, weight at dosing: 22-34 g, source: Breeding facilities at JAI Research Foundation, India, acclimation period: 5 days, diet: mice pellet diet (Amrut brand), Nav Maharashtra Chakan Oil Mills Ltd, India, ad libitum water: drinking water (filtered through Aquaguard water filter system), ad libitum, housing: animals were housed 6 per cage in polypropylene cages with stainless steel grid top, with rice husk as bedding, number of animals per dose: 6 male rats/group, test compound concentrations: 0, 1.5, 3.0 and 6.0 mg /kg bw, dose volume: 10 mL/kg bw tests were carried out from 10.10. to 23.11.1998, Animals received the test compound by oral gavage application (gavage) for two consecutive days. Control group received only peanut oil, whereas mice from positive control received a single application of Mitomycin-C dissolved (4 mg/kg bw) in distilled water. The application volume was 10 mL/kg bw. For bone marrow preparation animals were sacrificed by cervical dislocation 24 hours after dosing. Femora were excised and epicondyle tips removed. Bone marrow was harvested in foetal calf serum. Smears prepared from the bone marrow serum was place on a slide and stained with 5 % Giemsa in phosphate buffer for 10 minutes. The incidence of micronuclei in polychromatic and normochromatic erythrocytes among 1000 polychromatic erythrocytes (PCE), normochromatic erythrocytes (with and without micronuclei) and the ratio of PCE to normochromatic erythrocytes (NCE) (P/N) was determined for each animal. Statistical methods: Student's t-test.

Findings:

No deaths occurred. Clinical signs (tremor, polyurea, and piloerection) were observed after dosing at 6 mg/kg bw. There were no significant decreases in mean body weight change for any of the treated groups or control groups (vehicle and positive control). There were no statistically significant changes in the PCE/NCE ratio (see Table B.6.4-6). There was no relevant difference in the number and frequency of MPCEs between treated and vehicle control groups (see Table B.6.4-7). The number and frequency of MPCEs in the positive control group fulfilled the criteria of a positive response and demonstrated the sensitivity of the test system.

Table B.6.4-6: Summary of micronucleus results in male mice (PCE/NCE ratio data)

Harvest time (h)	Sex	No. of animals	Mean PCE/NCE ratio \pm standard deviation				
			Vehicle control #	1.5 mg/kg bw	3.0 mg/kg bw	6.0 mg/kg bw	Positive control #
24	Male	6	1.31 \pm 0.24	1.53 \pm 0.37	1.28 \pm 0.26	1.55 \pm 0.32	2.13 \pm 3.26

vehicle control, peanut oil (10 mL/kg bw per os); positive control, mitomycin-C (4 mg/kg bw i.p.)

Table B.6.4-7: Summary of micronucleus results in male mice

Harvest time (h)	Sex	No. of animals	Mean MPCE/total erythrocytes (%) \pm standard deviation				
			Vehicle control #	1.5 mg/kg bw	3.0 mg/kg bw	6.0 mg/kg bw	Positive control #
24	Male	6	0.06 \pm 0.06	0.03 \pm 0.03	0.05 \pm 0.05	0.07 \pm 0.05	0.93 \pm 0.43*

vehicle control, peanut oil (10 mL/kg bw per os); positive control, mitomycin-C (4 mg/kg bw i.p.)

* significant at 5 % level ($p \leq 0.05$)

Conclusion:

Aluminium phosphide technical does not have micronucleus induction potential in mice up to a dose of 6 mg/kg bw oral gavage (one-half of LD₅₀).

Report: Barbosa, A. et al. (1994): Determination of genotoxic and other effects in mice following short term repeated-dose and subchronic inhalation exposure to phosphine, Published paper, Environ. Mol. Mutagen. 24:81-88 (1994) (TOX97-50676)

Guidelines: Not indicated

Deviations: A positive control was not included in this study.

GLP: No

Acceptability: The study is considered to be supplementary.

Material and methods:

Common name: hydrogen phosphide, phosphine, PH₃, code no.: none, lot/batch no.: not specified, purity: not specified, description: clear, colourless gas (not specified in the paper), negative/solvent control: room air, test organism/cells: mouse bone marrow erythrocytes, spleen lymphocytes, peripheral blood and skin keratinocytes, species: mouse, strain: Balb-C-mice, Source: SPF Biological Facility, University of New South Wales, Australia, age at dosing: subchronic: 42 - 49 day old, short term: 50 - 54 day old, weight at dosing: subchronic: males (19.5 - 22.4 g), females (15.5 - 17.4 g), short term: males (19.9 ± 3.6 g), females (17.8 ± 1.2 g), diet: Rat & mouse standard ration (code RM-S, Lab-Feed, Australia), water: filtered water ad libitum, housing: 45 cm x 25 cm x 13 cm polypropylene cages with a wire lid and cellulose based bedding. Environmental conditions during non-exposure periods:

Temperature:	21 - 23 °C
Humidity:	50 - 60 %
Air changes:	Not specified
Photoperiod:	12 hours light: 12 hours dark
Dose levels:	
Subchronic inhalation exposure:	0, 0.3, 1.0 and 4.5 ppm
Short-term repeated dose inhalation exposure:	5.5 ppm

Phosphine gas was procured at concentration of 1400 ppm phosphine (0.14 % v/v) in nitrogen. Dose selection for the subchronic exposure was based on the current ACGIH (American Conference of Governmental Industrial Hygienists). Threshold limit Value Time Weighted Average (TLV-TWA) (0.3 ppm), TLV-Short Term Exposure Limit (1.0 ppm) and a representative of the maximum tolerated dose (4.5 ppm). The concentration used in the short-term study (5.5 ppm) was selected as a representative of the maximum tolerated dose. Subchronic test: Four groups of 12 male and 12 female mice each were used in this treatment, where animals were exposed to phosphine concentrations of 0, 0.3, 1.0 and 4.5 ppm, for a period of 13 weeks, 5 days/week, 6 hours/day. Animals were marked and weighed individually at the commencement of the experiment and re-weighed just prior to sacrifice, 15 hours after the last exposure. Individual organ weights (kidneys, lungs, liver, heart, brain and spleen) were obtained at necropsy and are reported as a percentage of respective final whole body weights. Bone marrow polychromatic erythrocytes (PCE) were mounted on slides, stained and scored. The frequency of micronucleated cells was recorded in a minimum of 1000 PCE per animal. The ratio of PCE to 400 normochromatic erythrocytes (NCE) was determined in order to assess chemical-induced haematopoietic toxicity. Spleen lymphocytes were stimulated to divide by addition of concanavalin-A (Con-A, 2.5 µg/mL), and cytokinesis

was blocked by the addition of 4.5 µg/mL cytochalasin B at 21 hours of culture. Cells were harvested at 48 hours. Micronuclei were scored in a minimum of 1000 binucleated (BN) cells. Point mutation assays were also performed on spleen lymphocytes. Cells lacking HPRT activity were selected using thioguanine. The unselected cells were plated at 10 cells/well and the selected cells at 5×10^4 /well. The mutants were quantified using the ratio of cloning efficiencies obtained with and without thioguanine selection. Short-term test: 10 male and 10 female mice were used in this 2-week treatment. Six males and 6 females were exposed to 5.5 ppm of phosphine for 5 days/week, 6 hours/day while 4 males and 4 females were exposed to room air. Animals were weighed individually 1 hour before the start of the first exposure and 15 hours after the last exposure. The endpoints evaluated were weight gain, MN incidence in PCE from whole blood and in cultured skin keratinocytes. Skin keratinocytes from the exposed mice, cultured appropriately to produce binucleate cells, were fixed, prepared onto slides and stained with 4 % Giemsa. At least 1000 binucleated keratinocytes were scored per animal. Smears of whole blood were prepared on clean glass slides, air dried and stained with Diff-Quik Stain Set. Micronucleated cells were scored in 1000 – 2000 PCE/mouse. Statistical methods: In the subchronic study, multiple linear regression techniques were used to analyse weight gain and HPRT mutation frequency in spleen lymphocytes. Differences between sexes and between doses of phosphine in the induction of MN/PCE in bone marrow were examined by two-factor analysis of variance using the General Linear Models (GLM) procedure. An unpaired t-test was used to analyse differences in relative organ weights and MN in spleen lymphocytes. In the short-term repeated-dose study, weight gain was analysed by GLM and frequencies of MN in peripheral blood and skin keratinocytes were analysed using an unpaired t-test.

Findings:

Subchronic study (13 weeks exposure at 0, 0.3, 1.0 and 4.5 ppm): Male and female mice exposed to 4.5 ppm of phosphine showed signs of itching around the face, tail and feet during exposure and were less active at the end of each exposure than the other groups. No other clinical signs were observed. There was a highly significant decrease in weight gain, which was dose related in both sexes, with a greater effect in females ($P < 0.0001$) (see Table B.6.4-8). Organ weights relative to bodyweight showed statistically significant increase in treated females: lung and heart at 0.3, 1.0, and 4.5 ppm, liver and spleen at 1.0 and 4.5 ppm, kidney at 4.5 ppm (Table B.6.4-9). This was not confirmed in males, which showed occasional statistically significant reductions unrelated to concentration of phosphine. This pattern of relative organ weights in males and females may be due in parts to the variations in bodyweight gain than a real increase or decrease in organ weights. No evidence of toxicity was noticed as indicated by the percentage PCE, which was, in excess of 40% at all exposure levels (see Table B.6.4-10). MN frequency in bone marrow PCEs did not differ from control values ($P > 0.9$) at dose levels of 0.3 and 1.0 ppm, but was 111 % higher than control values (5.8 vs. 2.6) at a dose of 4.5 ppm in female mice. A significant elevation in MN frequency in spleen lymphocytes was observed in both sexes (unpaired t-test, $P < 0.05$) at 4.5 ppm (the intermediate groups were not assessed). Analysis of HPRT mutation frequency in spleen lymphocytes showed no significant differences between the sexes or between control (6.75×10^{-6}) and exposed groups (4.42×10^{-6} , 6.30×10^{-6} , 5.25×10^{-6} for 0.3, 1.0 and 4.5 ppm respectively). Short-term study (2 weeks exposure at 0 and 5.5 ppm): Exposed male and female mice had a slightly lower weight gain than controls, but this was not statistically significant. There were no significant differences in the MN frequency in PCE from peripheral blood or in skin keratinocytes.

Table B.6.4-8: Weight gain and percentage weight gain in mice following 13 weeks exposures to phosphine

PH ₃ concentration (ppm)	Females (10/concentrations)			Males (10/concentrations)		
	Body Wt ^a	Wt gain	% Wt gain ^b	Body Wt ^a	Wt gain	% Wt gain ^b
0	20.1 ± 2.1	3.38 ± 0.57	20.1 ± 3.1	26.6 ± 1.7	4.20 ± 0.45	17.0 ± 1.4
0.3	18.5 ± 1.5	2.82 ± 0.42	18.1 ± 2.6	25.5 ± 1.3	3.34 ± 0.51	15.1 ± 2.5
1.0	19.4 ± 0.7	2.83 ± 0.37	17.2 ± 2.5	25.5 ± 2.3	3.11 ± 0.47	14.1 ± 2.5
4.5	18.0 ± 1.9	1.77 ± 0.32	11.0 ± 1.7	25.5 ± 2.5	2.92 ± 0.70	12.9 ± 2.6

a Terminal body weight; all data are mean ± S.D.

b Highly significant decrease related to exposure (p < 0.0001)

Table B.6.4-9: Organ weights as a percentage of whole body weight, and absolute organ weights, in mice following 13 weeks exposure to different concentrations of phosphine

Group (sex)	1M	2M	3M	4M	1F	2F	3F	4F
Target dose [ppm]	0	0.3	1	4.5	0	0.3	1	4.5
Body weight At termination (g)	26.6	25.5	25.5	25.5	20.1	18.5	19.4	18.0
Organ weights								
Brain [g]	0.40	0.38	0.37	0.41	0.38	0.36	0.38	0.38
Brain [% bodyweight]	1.57	1.41 ^c	1.46	1.62	2.01	1.93	1.98	2.16
Heart [g]	0.20	0.17	0.19	0.17	0.12	0.11	0.12	0.12
Heart [% bodyweight]	0.78	0.63 ^c	0.76	0.67 ^b	0.53	0.61 ^b	0.62 ^c	0.65 ^c
Lungs [g]	0.17	0.16	0.16	0.16	0.13	0.13	0.13	0.13
Lungs [% bodyweight]	0.65	0.60 ^b	0.62	0.63	0.64	0.72 ^c	0.69 ^c	0.74 ^c
Liver [g]	1.24	1.28	1.13	1.22	0.98	0.88	0.80	0.95
Liver [% bodyweight]	4.77	4.73	4.41	4.86	4.88	4.76	4.55 ^b	5.40 ^b
Kidney [g]	0.41	0.39	0.40	0.39	0.28	0.22	0.24	0.23
Kidney [% bodyweight]	1.59	1.45 ^c	1.55	1.53	1.19	1.25	1.24	1.30 ^b
Spleen [g]	0.08	0.07	0.08	0.07	0.07	0.07	0.08	0.08
Spleen [% bodyweight]	0.30	0.26	0.30	0.28	0.36	0.37	0.41 ^c	0.45 ^c

b Statistically significant compared with respective controls, 0.01 < p < 0.05

c Statistically significant compared with respective controls, p < 0.001

Table B.6.4-10: Frequency of micronuclei in PCE from bone marrow and lymphocytes from spleen in mice following 13 weeks exposure to different concentrations of phosphine

Phosphine Conc. (ppm)	Bone marrow					Spleen	
	Number of animals	Total MN scored	Total PCE scored	% PCE	MN/1000 PCE Mean \pm S.D. ^a	MN/1000 ^b BN cells	Total BN scored
Males							
0	10	67	13500	49.0	4.7 \pm 2.1	3.3 \pm 1.0	5618
0.3	6	26	6500	49.5	3.8 \pm 2.4	N.A.	
1.0	6	17	6200	40.7	3.4 \pm 1.2	N.A.	
4.5	10	109	19600	44.2	5.5 \pm 2.0	6.3 ^d \pm 1.6	6000
Females							
0	10	29	11600	48.2	2.6 \pm 1.0	3.4 \pm 1.3	7000
0.3	6	20	6000	49.1	3.3 \pm 1.0	N.A.	
1.0	6	24	6300	43.3	3.7 \pm 1.7	N.A.	
4.5	10	117	19200	46.7	5.8 \pm 1.7	7.5 ^d \pm 1.3	4000 ^e

a Calculated from individual frequencies of MN/1000 PCE

b Five animals/group.

c Not assessed

d Statistically significant compared with respective control, $p < 0.005$

e Insufficient cells available to score 1000 cells/animal.

Conclusion:

Following the subchronic inhalation exposure of mice for 13 weeks, phosphine at the highest test concentration of 4.5 ppm caused an increase in micronuclei frequencies in bone marrow polychromatic erythrocytes and spleen lymphocytes. However, no increase were observed in gene mutations at the HPRT locus in the recovered spleen lymphocytes. Short-term exposure of mice to 5.5 ppm over two weeks did not affect micronucleus frequency in polychromatic erythrocytes from peripheral blood or in skin keratinocytes.

Report:

McKeon, M.E. (1993): Genotoxicity test on phosphine in the in vivo/in vitro assay for unscheduled DNA synthesis in rat primary hepatocyte cultures at two timepoints, report-no.: A0040-0-494, Metal phosphide task force, Hazleton Washington, Inc., 9200 Leesburg Pike, Vienna, Virginia 22182, USA, 2 July 1993 (TOX2005-285)

Guidelines:

US EPA FIFRA 84-2

Deviations:

None

GLP:

Yes

Acceptability:

The study is considered to be acceptable.

Material and methods:

Hydrogen phosphide, phosphine, PH₃, code no.: none, lot/batch no.: container #1 = 864-2241-04, container #2 = 864-2241-01, container #3 = 864-2241-03, container #4 = 864-2241-02, purity: 99.98 %, description: colourless gas, negative/solvent control: room air, positive controls: dimethyl nitrosamine at 10 mg/kg and 15 mg/kg, test organism/cells: rat liver cells, species: rat, strain: fisher 344, source: Charles River Breeding, Inc., USA, acclimatisation period: at least 10 days, age at dosing: 12 weeks, weight at dosing: 218 – 253 g., diet: purina certified rodent chow 5002, water: tap water, ad libitum, housing: 2 per cage in suspended stainless steel wire mesh cages during acclimatisation, individually thereafter during non-exposure periods.

Environmental conditions during non-exposure periods:

Temperature:	21 ± 1 °C
Humidity:	42 ± 9 %
Air changes:	Not specified
Photoperiod:	12 hours light : 12 hours dark
Dose levels, test compound:	target levels of 5, 15, 20 and 25 ppm
Mean analysed exposure concentrations of	4.8, 13, 18 and 23 ppm
Positive compound:	10 mg/kg and 15 mg/kg

Dose preparation: aerosol generation: chambers were supplied with room air mixed with phosphine from a gas cylinder, regulated by a metering valve and monitored by a mass flowmeter. The main exposure parameters are shown in Table B.6.4-11:

Table B.6.4-11: Main exposure parameters

Exposure period	6 hours
Exposure chamber volume	1000 l
Chamber air flow	201 – 218 litres/minute
Air changes	12/hour
Actual chamber temperature	21 ± 1 °C
Actual relative humidity range	40 ± 7 %
Target concentration	5, 15, 20 and 25 ppm
Nominal concentration	4.7, 14, 20 and 24 ppm
Mean analysed concentration	4.8, 13, 18 and 23 ppm

Groups of ten male rats were given a single, six-hour, whole body inhalation exposure to phosphine at target levels of 5, 15, 20 and 25 ppm. A negative control group consisting of 6 male rats received room air only. Six more animals were dosed with the positive control, DMN, by intraperitoneal injection. The actual concentration of phosphine in the chambers during the exposure period was determined by withdrawing chamber air at 90-minute intervals during the exposures and analysing by gas chromatography. Airflow, temperature, relative humidity and static pressure were measured at half hour intervals. Animals were observed for pharmacologic and/or toxicologic signs during exposure, post exposure and prior to sacrifice. Observations included body weights and physical observations. Two time-points for UDS were employed, one approximately 2 to 3 hours and another 12 to 14 hours after exposure. Hepatocytes from at least three rats at each exposure level and each timepoint were prepared for UDS analysis. After hepatocyte harvesting, radiolabelling with thymidine and the preparation of autoradiographs, the net nuclear grain counts from treated animals were compared with the control counts. Net nuclear grain count was determined from 150 nuclei per animal (50 per coverslip, 3 coverslips per animal). The test material is considered active in the UDS assay at doses that cause an increase in the group average of mean net nuclear grain count to at least five grains per nucleus above the concurrent negative control average, leading to a positive number and/or the group average of the percent of nuclei with five or more net grains to increase at least 10 % above the average of the concurrent negative controls. The test material is considered inactive in this assay if none of the above conditions are met in any of the treated animals. When results are neither clearly positive nor clearly negative, dose response, frequency, distribution and reproducibility among animals is considered. The criteria for a positive response are based on a statistical analysis of the historical data and calculation of the minimum increase required for a significant UDS response as described by Casciano and Gaylor (Casciano, D.A and Gaylor D.W. Statistical criteria for evaluating

chemicals as positive or negative in the hepatocytes DNA repair assay. Mutation Research, 122 p 81-86, 1983).

Findings:

At 18 and 23 ppm laboured breathing was observed immediately following exposure. Respiration returned to normal in many animals by 2 hours post exposure. Slight decrease in body weight was observed at 13, 18 and 23 ppm. There was no evidence that phosphine caused increased unscheduled DNA synthesis in liver cells of male rats exposed by inhalation to levels up to 23 ppm. Mean net nuclear grains and % cells with more than 5 grains were comparable among all treated animals and air-exposed controls. Positive control animals treated with DMN showed marked increases in nuclear labelling that greatly exceeded both criteria used to indicate UDS.

Conclusion:

Hydrogen phosphide showed no evidence of causing increased unscheduled DNA synthesis in hepatocytes of male rats exposed by inhalation to levels up to 23 ppm.

Report: Kligerman, A.D. et al.(1994), Cytogenetic effects of phosphine inhalation by rodents. I: acute 6-hour exposure of mice, Published paper. Environ. Mol. Mutagen. 23:186-189 (1994) (TOX97-50677)

Guidelines: Guideline not specified

Deviations: A positive control was not included in this study
Samples of bone marrow were taken only once at 20 hours after treatment.

GLP: No

Acceptability: The study is considered to be supplementary.

Materials and methods:

Phosphine (750 ppm in nitrogen); batch no.: not stated; purity: 99.99 %; five male CD-1 mice (age: approx. 12 weeks) per group were exposed to target concentrations of 0, 5, 10 and 15 ppm phosphine in nitrogen for 6 hours in a whole-body inhalation experiment. 24 hours after termination exposure, bone marrow smears were prepared, and spleens were removed, macerated and splenocytes cultured for the analysis of chromosomal aberrations (CA), sister chromatid exchanges (SCE) and micronucleus (MN) formation. Inhalation atmospheres were generated by metering PH₃ in nitrogen mixture through a mass flow controller into the chamber airflow stream. The inhalation system is characterised in Table B.6.4-12:

Table B.6.4-12: Characterisation of the inhalation system

Chamber volume:	133 L	Analytics:	IR Spectroscopy
Airflow rates:	35 L/min	Chamber temperature :	23.1 – 23.8 °C
Chamber volume change:	15.8 per hour	Relative humidity:	64 – 68 %
Oxygen content:	> 20.3 %		

Findings:

The actual concentrations measured were 0, 5.24 ± 0.69 , 9.94 ± 0.69 and 16.00 ± 1.15 ppm, respectively. No increase in any of the cytogenetic endpoints was found at any of the concentrations examined. The only statistically significant response was a concentration related slowing of the cell cycle in the splenocytes.

Conclusion:

There is no evidence that phosphine is clastogenic, aneuploidogenic or capable of inducing SCE under the conditions of the test.

Report:

Kligerman, A.D. et al. (1994): Cytogenetic and germ cell effects of phosphine inhalation by rodents: II. Sub-acute exposure to rats and mice; Environ. Mol. Mutagen. 24: 301 – 306 (TOX2002-830)

Guidelines:

Not indicated

Deviations:

In the OECD guideline 474 sampling times for bone marrow shorter than 24 h post exposure are not recommended.

GLP:

No

Acceptability:

The study is considered to be supplementary.

Materials and methods:

Test material: phosphine (21500 ppm in nitrogen, AGA Specialty Gas. Inc., Maumee, OH); batch no.: not stated; purity: no data. Five male B6C3F1 mice and 5 male F344/N rats (age: approx. 8 weeks) were exposed to target concentrations of 0, 1.25, 2.5 and 5 ppm phosphine in nitrogen for 6 hours/day for 9 days over a 11-day period in a whole-body inhalation experiment. 18 - 20 hours after termination exposure, bone marrow smears were prepared from rats and peripheral blood smears were made from mice and rats. In mice, isolated mononuclear leucocytes were analysed for sister chromatid exchange (SCE); chromosomal aberrations (CA) were determined in peripheral blood cells (PBL), and micronucleus (MN) formation in binucleated (BN) lymphocytes and polychromatic erythrocytes (PCE). Bone marrow smears of rats were analysed for micronucleated PCEs, and peripheral blood was investigated for SCE and CA. Inhalation atmospheres were generated by metering PH_3 in nitrogen through a mass flow controller into the chamber airflow stream. The inhalation system is characterised in Table B.6.4-13.

Table B.6.4-13: Characterisation of the inhalation system

Chamber volume:	133 L	Analytics:	IR Spectroscopy
Airflow rates:	35 L/min	Chamber temperature:	23.1 – 23.8 °C
Chamber volume change:	15.8 per hour	Relative humidity:	64 – 68 %
Oxygen content:	> 20.3 %		

Findings:

Phosphine inhalation caused no statistically significant increases in the incidences of SCE, CAs in peripheral blood cells, MN in peripheral blood and in binucleated lymphocytes of mice. Cytogenetic results in rats were similar to those obtained in mice.

Conclusion:

No evidence of SCE, CA or MN induction was found in peripheral blood and bone marrow cells. The vast majority of chromosomal aberration were simple chromatid or chromosome deletions. Therefore, there is no evidence that phosphine causes cytogenetic damage in mice or rats under the conditions of this test.

Report:	Pal, B.B.; Bhunya, S. P. (1995): Mutagenicity testing of a rodenticide, zinctox (zinc phosphide) in a mouse in vivo system; In Vivo 9, 81-83 (TOX2002-183)
Guidelines:	Not stated.
Deviations:	Only 4 animals per group were tested, lack of a positive control and lack of standardisation concerning dose regimen.
GLP:	No
Acceptability:	The study is considered to be not acceptable.

Material and Methods:

Test Material:	zinc phosphide ("zinctox")
Description:	solid
Lot/Batch no.:	not stated (Source: All India Medical Corporation, Bombay)
Purity:	80 %
Control materials:	
Negative:	vehicle (water) only
Solvent/vehicle:	double distilled water
Positive:	none
Test animals:	
Species:	mouse
Strain:	inbred Swiss mice
Age:	10-12 weeks
Weight at dosing:	25-30 g
Source:	not stated
Number of animals per dose:	4 animals

Test compound concentrations: 20, 30 and 40 mg/kg bw (micronucleus assay, chromosome aberration assay and sperm abnormality assay); 8 mg/kg bw for 5 consecutive days (chromosome aberration assay). Bone marrow chromosome aberration assay: For the acute experiment, 4 animals per group were treated with different doses (20, 30 and 40 mg/kg bw) by different routes (i.p., p.o. and s.c.) for exposure periods of 6, 24 and 48 hours. In the sub-acute experiment, 4 mice received repeated i.p. injections of 8 mg/kg bw every 24 hours for 5 consecutive days. 24 hours after the last injection animals were sacrificed, cytological slides prepared, and 75 good metaphase spreads were scored for each animal. Micronucleus assay: Doses of 20, 30 and 40 mg/kg bw were injected twice i.p. at an interval of 24 hours to groups of 4 animals. Mice were sacrificed 6 hours after the last injection, and bone marrow smears were prepared. 1000 each of polychromatic erythrocytes (PCE), normo-chromatic erythrocytes (NCE) and nucleated white cells were scored per animal. Sperm abnormality assay: Doses of 20, 30 and 40 mg/kg bw were injected i.p. at an interval of 24 hours on 5 consecutive days to groups of 4 animals. Mice were sacrificed 35 days after the first injection. Sperm smears were drawn after collection from the caudae epididymides in isotonic saline, fixed in methanol and then stained in 2 % eosin-y. 500 sperms per animal were examined for

abnormalities. Evaluation criteria: Statistically significant increase of the percentage of abnormal sperm in comparison to the controls.

Findings:

The percentage of micronucleated PCEs and NCEs combined and nucleated cells was dose-dependently and statistically significantly increased. In contrast, the chromosomal aberration assay did not yield a dose-related trend of the observed effects. However, the usefulness of the test is limited due to the lack of a positive control. In addition, there are several shortcomings (number of animals tested, inconsistencies in the dose regimen), and the fact that the bioavailability of zinc phosphide from s.c. and i.p. administration may be considered questionable.

Different types of abnormal sperm heads including blunt hook, snake hood, beak, amorphous, bell calyx etc. were observed in the sperm abnormality assay. Percentage of abnormal sperm increased with increasing doses. In the opinion of the authors, zinc phosphide indicated a genotoxic potential in mice in view of the statistically significant higher frequencies of abnormal sperm observed at different doses. However, current guidelines on genotoxic effects on germ cells focus on cytogenetic effects, and not on alterations in sperm morphology.

Conclusions:

No positive control was used in the test. In addition, there are several shortcomings (number of animals tested, inconsistencies in the dose regimen), and the fact that the bioavailability of zinc phosphide from s.c. and i.p. administration may be considered questionable. The results of the sperm abnormality assay are not useful for the evaluation of genotoxicity. Therefore, the study is considered to be not acceptable.

Report:	Rajesh Sundar, S. (1999): Dominant lethal test of aluminium phosphide technical in mice. JAI Research Foundation (JRF), Gujarat, India, JRF study No. 370, 07.07.1999 (TOX2006-224)
Guidelines:	OECD 478
Deviations:	No positive control was tested. Only one dose level was tested. Test substance purity not indicated.
GLP:	No
Acceptability:	The study is considered to be supplementary.

Materials and methods:

Aluminium phosphide techn., lot/batch: not reported, purity: not reported, solvent used: peanut oil.

Control materials: negative control: peanut oil, solvent: peanut oil, positive control: not conducted.

Test animals: species: mice, strain: Swiss albino, age: not reported, weight at dosing: 29-42 g, source: Hafkine Bio-pharmaceutical Corporation Ltd., Mumbai, India, acclimation period: 5 days, diet: mice pellet diet (Amrut brand, Nav Maharashtra Chakan Oil Mills Ltd, India) ad libitum, water: drinking water (filtered through Aquaguard water filter system), ad libitum, housing: animals were housed 4 per cage in polypropylene cages with stainless steel grid top, with rice husk as bedding, number of animals per dose: 11 and 10 male mice in the treated and control group, respectively.

Environmental conditions: temperature: climatically controlled, humidity: climatically controlled, air changes: not reported, photoperiod: alternating 12 h light and dark cycles
test compound concentrations: 0 and 6 mg/kg bw.

Male mice were administered aluminium phosphide at a dose level of 6 mg/kg bw. The test substance was suspended in peanut oil and the dose volume maintained at 10 mL/kg bw.

Animals of the control group received plain peanut oil.

Three untreated virgins were cohabited with one treated male for 7 days. Females were then moved to another cage and another set of 3 females were cohabited. This procedure was followed by 8 consecutive weeks.

The females were sacrificed during 16th to 18th day after cohabitation. Both uterine horns were examined for the number of live implants, the number of dead implants including early death (moles) and late death (dead fetuses) and total number of implants.

The pre-implantation loss was estimated by comparing the total number of implants per pregnant female of the treated group with those of the control group. Post implantation loss was calculated by determining the ratio of dead to total implants in treated and control groups. Dominant lethality was calculated by comparing the number of live implants per female in the treated group to those in the control group. Statistical methods: Student's t-test.

Findings:

No deaths occurred. Lethargy was observed in males up to 3 days after dosing at 6 mg/kg bw. Significant decreases in body weight were noted in males during the first 2 weeks after dosing at 6 mg/kg bw.

No statistically significant change in pre-implantation loss was noted, but there was a significant increase in post-implantation loss in the first and third week in the 6 mg/kg bw group. A significant reduction in the number of live implants was observed in the 6 mg/kg bw group during the first and third week. For details see Table B.6.4-14.

Table B.6.4-14: Results of dominant lethal assay in mice

Dose (mg/kg bw)	Parameter	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8
0 (control)	Pregnancy frequency (%)	66.7	46.7	63.3	66.7	60.0	73.3	63.3	80.0
6		69.7	57.6	72.7	60.6	63.6	42.4	54.5	42.4
0 (control)	No. live implants (mean values)	10.2	11.1	11.0	10.9	10.9	10.2	10.2	9.6
6		7.1*	9.5	8.3*	10.0	9.5	8.6	8.2	8.0
0 (control)	No. total implants (mean values)	10.6	11.2	11.5	11.2	11.5	10.6	10.5	10.2
6		9.0	9.7	10.6	10.0	10.2	10.0	9.7	9.4
0 (control)	Post-implantation loss (mean values)	0.04	0.01	0.04	0.03	0.05	0.04	0.04	0.06
6		0.23*	0.05	0.21*	0.01	0.07	0.22	0.17	0.10

* significant at 5 % level ($p \leq 0.05$)

Conclusions:

Increase in post implantation loss and reduction in the number of live implants was observed at the only dose level of 6 mg/kg bw. Parental toxicity (decreased body weight of the males) was also observed in this dose group.

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

Phosphine was assessed for chronic inhalation toxicity and carcinogenicity in a combined 104 week study in rats (summary in Table B.6.5-1). The gas was produced by aluminium phosphide. In the inhalation study, body weight, food consumption, routine haematology, serum biochemical, and urinary analyses were all similar to control animals.

Ophthalmological observations, gross pathology, organ weights and histopathology indicated no adverse effects from phosphine exposures. The NOAEL was 3.0 ppm, the highest concentration tested. This dose level is equivalent to 1.1 mg/kg bw/day.

In two older limited dietary studies, rats received diets treated with phosphine released from aluminium phosphide. Behaviour, general appearance, survival, body weight, food consumption, haematology, blood chemistry, urine analyses and bone smear data, as well as gross and microscopic findings and rate of tumour development, did not reveal any toxic effects from the aluminium phosphide treated diet. However, the test design of both studies was insufficient. Therefore, the oral studies are considered to be not acceptable.

Based on lack of exposure and the absence of genotoxic concern waiving of a long term /carcinogenicity study in a second species was seen as justified.

Table B.6.5-1: Summary of long-term toxicity and carcinogenicity

Study and dose levels (mg/kg/day)	NO(A)EL	LOEL	Reference
Combined rat chronic (2 year) toxicity and carcinogenicity study, 0, 0.3, 1, and 3 ppm by inhalation with purified PH ₃	Toxicity: NOAEL: 3 ppm equivalent to 0.0042 mg/L or 1.1 mg/kg bw/day	Toxicity: LOEL: > 3 ppm Based on lack of systemic toxicity at any dose level	Newton, 1998 (TOX2000-98)
	Carcinogenicity: NOEL: 3 ppm	Carcinogenicity: LOEL: > 3 ppm based on lack of carcinogenicity at any dose level	
Rat chronic (2 year) toxicity, oral, levels of phosphine in diet after fumigation ranged from 0.167-7.5 mg/kg	No effects observed. However, the study is considered to be not acceptable.	-	Hackenberg, 1972/1969 (TOX96-52058) / (TOX2005-286)
Rat chronic (2 year) toxicity, oral, level of phosphine in diet after fumigation 5 ppb	No effects observed. However, the study is considered to be not acceptable.	-	Telle et al., 1985 (TOX2002-831)

B.6.5.1 Long-term (2 years) inhalation toxicity – rat

Report: Newton, P.E. (1998): 2-Year combined inhalation chronic toxicity and oncogenicity study of phosphine in rats. MPI Research, Mattawan, USA; unpublished report no. 750-001, 10.09.1998 (TOX2000-98)

Guidelines: Guidelines of the Environmental Protection Agency (USA): Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Section 83-5

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Report: Newton, P.E. et al.(1999): A 2-year inhalation study of phosphine in rats, Inhalation Toxicol. 11, pp. 693-708, 1999 (TOX2002-189)

Materials and methods:

Test material: Phosphine; purity: 1 % in nitrogen. Phosphine was administered as a gas to rats via whole-body exposure 6 hours per day, generally 5 days per week, for 104 consecutive weeks. Three groups (Group 2, 3 and 4) each consisting of 60 male and 60 female Charles River Fischer CDF (F-344)/CrI/BR VAF/Plus rats were exposed to target phosphine exposure levels of 0.3, 1 and 3 ppm of phosphine, respectively. Another group of 60 males and 60 females served as negative control and was exposed to clean air. Exposure levels were determined using gas chromatography. Exposure commenced on March 5, 1996 and terminal necropsies were conducted on March 4 though 10, 1998.

Each rat was observed twice daily for mortality and signs of toxicity. Detailed clinical observations of each animal were conducted weekly. Individual body weights were recorded weekly during the study and then at approximately monthly intervals. After 26, 52, 78 and 104 weeks of exposure, various haematologic, clinical chemistry and urinalysis evaluations were conducted. Ophthalmoscopic observations were conducted prior to exposure and prior to the 52 week interim sacrifice and 104 week terminal sacrifice.

After 52 weeks of exposure, 10 rats per sex per group were euthanised and received a complete necropsy. Tissues were collected, organs weighed and tissues preserved for shipment to Experimental Pathology Laboratories, Inc. (EPL), Herndon, Virginia, for histopathologic evaluation. After 104 weeks of exposure, all survivors were euthanised and received a complete necropsy. Tissues were again collected, organs weighed and tissues preserved for shipment to EPL for histopathologic evaluation.

Through 104 weeks, the mean phosphine exposure levels were 0.3 ± 0.019 , 1.0 ± 0.037 and 3.01 ± 0.086 ppm.

Findings:

There was no apparent test article related effect seen in the detailed clinical observations. The findings recorded occurred with a low incidence and were sporadic.

Through 104 weeks of exposure, there were a total of 99 unscheduled deaths (7 males and 14 females at 0 ppm, 16 males and 15 females at 0.3 ppm, 14 males and 9 females at 1.0 ppm, and 12 males and 12 females at 3 ppm). These animals were either found dead, sacrificed in extremis, or died post bleeding. These deaths are not considered to be test article-related.

There was no apparent effect of the test article exposures on body weight. All exposure groups gained similar weight over the 52 week chronic and the 104 week oncogenicity exposure period.

There was no apparent effect of the test article exposures on food consumption. Statistically significant differences between the test article exposed groups and the control group were noted. However, these occurred sporadically, were not consistent over time, small in magnitude, and not considered to be test article-related or biologically significant.

The observations in ophthalmoscopic examinations were representative of pathology that would be expected for this group of animals considering age, sex, and strain; no obvious trends in pathology suggestive of test article-related reactions were observed. All rats demonstrated some degree of corneal superficial dystrophy.

There were no apparent test article related alterations in the haematology parameters evaluated. Three values for MCV were statistically different from controls in the 1 and 3 ppm

group males. Because these are calculated values and alterations did not occur in the erythrocyte counts or hematocrit, these changes considered incidental.

There were no apparent test article related alterations in the biochemistry parameters evaluated. Occasional values were statistically different from controls. A slightly higher chloride value occurred in the 3 ppm males at 1 interval. Because this occurred in only one sex, did not have a dose response, and the magnitude of difference compared to the controls was small and not biologically meaningful, this was considered incidental and not related to test article administration. A slightly lower creatine phosphokinase occurred in the 1 ppm group males at one interval. Because there was no dose response, this was considered incidental and not related to test article administration. A slightly lower cholesterol occurred in the 1 and 3 ppm group males at 1 interval. Because the values at this interval were similar to previous intervals and because the control males had an increase in mean cholesterol at this interval, these observations were considered incidental and not related to test article administration. Slightly higher alkaline phosphatase values occurred in the 0.3, 1, and 3 ppm group females at one interval. Because there was no dose response and values were similar to other intervals, and because the control females had a decreased mean at this interval, this observation was considered incidental and not related to test article administration. A slightly lower cholesterol occurred in the 0.3 ppm group females at 1 interval. Because there was no dose response, this observation was considered incidental and not related to test article administration.

There were no apparent test article-related alterations in the urinalysis parameters evaluated. A larger volume of urine was noted in the 3 ppm group males at 1 interval. A number of urine samples with high volumes and low specific gravity were noted in both males and females at this interval. Data points with the most extreme values were excluded from statistical evaluation; however, the observation of higher urine volume in the 3 ppm group males was considered incidental and not related to test article administration. At the 104 week interval, urine protein was quantitated for samples which had greater than or equal to 300 mg/dL protein by a semiquantitative method. There was no increase in urinary protein for rats exposed to the test article at 0.3, 1, or 3 ppm when compared to control rats.

No treatment related variations in organ or body weights were seen. The only statistically significant organ weight change in male or female rats treated with phosphine was decreased relative liver/body weight ratio in the 3 ppm group males at the terminal sacrifice. This change was attributed to a slightly higher mean body weight in this group compared to controls. The absence of a similar finding in females, as well as no corresponding findings in clinical pathology or microscopic evaluations, suggests that the trend for liver weights to decrease with dose, which was seen in the males, is coincidental and unrelated to phosphine exposure.

There were no histomorphologic tissue alterations attributable to exposure to the test article in any tissue examined. No tumors that were attributable to test article exposure were noted.

The only statistically significant increase seen was in adenomas of the Islet cells in the pancreas in male rats. The incidence in the 3 ppm (high exposure level) group was 5 %. The higher value was statistically significant for the Peto Test, but not for the Cochran Armitage Trend Test or the Fisher Exact Test. However, this higher percentage is within the historical range of 0 to 10 % seen at this facility and within the 0 to 20 % seen in the historical data published by Charles River. Therefore, this statistically significant increase is considered to be random and not test article-related.

Conclusion:

In conclusion, under the conditions of this study, there were no treatment related changes suggestive of a toxic or carcinogenic effect seen in rats following 52 weeks and 2 years of whole-body inhalation exposure to 0.3, 1 or 3 ppm phosphine. The NOAEL was 3.0 ppm, the highest concentration tested. This dose level is equivalent to 1.1 mg/kg bw/day.

B.6.5.2 Long-term (2 years) oral toxicity – rat

Report:

Hackenberg, U. (1972): Chronic ingestion by rats of standard diet treated with aluminium phosphide, published paper, Toxicol. Appl. Pharmacol. 23, 147-158, Institut für Industrielle und Biologische Forschung, Cologne, German Federal Republic, 1972 (TOX96-52058)

and

U. Hackenberg (1969): 2 years toxicity studies with Phostoxin-treated food on rats, Institut für industrielle und biologische Forschung, Köln, Germany, report no. A0187/012, 22.04.69

Guidelines:

No

Deviations:

Batch and purity not indicated. Low number of animals used (60). One dose level only. The dose was increased during the study. No dose level can be determined due to the great variability of PH₃ levels between batches. Batches were used for treatment for weeks despite the fact that feed analysis, which was performed only sporadically and is poorly documented, shows rapid decay of PH₃ levels in the fumigated diet which, presumably, has not been stored adequately. Limited clinical and histopathological assessment. Environmental conditions, statistics, age and acclimatisation period of rats were not reported

GLP:

No

Acceptability:

The study is considered to be not acceptable.

WARNING: This document forms part of an EC evaluation data package and should not be used for any other purpose. Registration must not be granted on the basis of this document.

Materials and methods:

Aluminium phosphide (Phostoxin pellets), lot/batch no.: not specified, purity: not specified, description: 3 g of Phostoxin pellets containing about 2 g of aluminium phosphide and release about 1 g of phosphine. The pellets also contain ammonium carbamate. Vehicle control: untreated diet. Test animals: species: rat, strain: Wistar, SPF bred, source: Charles River Breeding Laboratories Inc., Wilmington, Massachusetts, USA, weight at start dosing: males: 166 – 288 g, females: 150 – 216 g, diet: laboratory chow produced by Altromin GmbH “Alleindiät für Ratten und Mäuse” pulverised, B 0101, fine ground, water: tap water, ad libitum, housing: individually in wire grid cages. A total of sixteen 100 kg batches of treated diets were prepared. Eight Phostoxin pellets, corresponding to a concentration of 48 g/metric ton, were introduced into batches 1 and 2 and stored for 72 hr. Fifteen Phostoxin pellets, corresponding to a concentration of 90 g/metric ton, were introduced into batches 3 through 16. Batches 3 and 4 were stored for 48 hr and batches 5 through 16 for 72 hr before mixing. After fumigation, each batch was mixed and then the contents were aerated for 1 hour. Quantitative assays for phosphine concentration were performed after aeration on each batch according to the mercuric chloride method of White and Bushey (1944). Batches 1 through 3 were analysed at approximately 1, 5 and 8 week after aeration. Batches 4 through 16 were analysed about 3 – 10 days after aeration. Diet was provided to animals in feeding vessels which were re-filled twice a week. Each treated batch of diet was fed for 5 to 7 week, the unused portion being stored in closed containers. 30 male and 30 female rats received diets treated with aluminium phosphide as Phostoxin pellets at a level of 48 g/metric ton from week 1 through 16 and 90 g/metric ton from week 17 to 104. Another group of 30 males and 30 females served as the negative control and received untreated basal diet for the duration of the study. Weekly records of body weight, food consumption, physical appearance and behaviour were kept for each animal. Daily observations were made for mortality, general appearance and behaviour. Clinical laboratory determinations were performed prior to the start of treatment and at 2, 6, 12, 18 and 24 months of study; samples were taken from 3 sets of 5 males and 5 females in each group, as follows: haematology, blood glucose and urine (set 1), SGPT and urea (set 2) and prothrombin time (set 3). After 104 weeks of exposure, all survivors were euthanised. Observations were recorded at necropsy for each animal sacrificed at termination, as well as those animals, which died or where sacrificed in moribund condition during the study and received a complete necropsy. A full range of tissues were collected, organs weighed (liver, heart, spleen, thyroid, kidneys, adrenals) and tissues prepared for histological examination. Except for the eye lens, all tissues, preserved from 5 males and 5 females in each group sacrificed at 24 months of study were examined microscopically. Furthermore, all tissues or organs with macroscopically recognisable or questionable neoplasms were examined microscopically. The assessment of bone marrow smears was also performed on 5 male and 5 female rats per group sacrificed at termination.

Findings:

Levels of phosphine remaining in the treated diets after fumigation and aeration ranged from 0.167 mg/kg to 0.377 mg/kg for batches 1 and 2, and from 0.205 mg/kg to 7.50 mg/kg for batches 3 through 16. The average phosphine level was found to be 0.996 mg/kg for batches 3 through 16 assayed 3 to 10 days after treatment of the diet. These values include residues of the Phostoxin pellets. Survival was comparable between control and test groups. Percent survival at 106 week of study, and mean survival times, were as follows: control males, 60 %, 98.6 week; test males, 53.3 %, 92.5 week; control females, 53.3 %, 89 week; and test females, 50 %, 95.3 week.

The general appearance and behaviour of the test rats were comparable to that of the controls throughout the 24 months test period. As the study proceeded, and in particular during the last quarter, an increasing number of control and test animals developed signs usually found in rats of this age: coarse fur, weight loss, signs of various infections, diarrhoea, disequilibrium and tumours. Mean body weights and weight ranges and food consumption were comparable in the first year of study between control and test level male and female rats. During the second year, a slight trend toward higher body weight and food consumption were observed among the test females as compared to the control females. Body weights and food consumption were similar between male test level and control animals in the second year of study. All blood chemical values, with the exception of a high serum urea concentration in the control males at 24 months, were within normal range. The haematologic determinations were within normal range for the control and test groups, and parallel trends were observed in both groups. Urine analyses revealed no meaningful differences between control and test groups. There were no meaningful differences in organ weights in control and treated animals that could be attributed to treatment. Rats which died during the test and those sacrificed at 24 months exhibited comparable gross and microscopic changes, and distinct changes attributable to the experimental regimen were not observed. The incidence of neoplasms between control and treated animals was not significantly different and was considered to represent the spontaneous tumour rate.

Conclusions:

Under the conditions of this study, there were no treatment related changes suggestive of a toxic or carcinogenic effect seen in rats exposed for 2 years to diet fumigated with phosphine generated by incorporation of Phostoxin pellets (aluminium phosphide and ammonium carbamate) at 48 g/metric ton (weeks 1 – 16) and 90 g/metric ton (weeks 17 onwards). However, the test design was insufficient and the study was considered to be not acceptable.

Report:	Telle, A.-M. et al (1985): Nutritional and toxicological effects of long term ingestion of phosphine fumigated diet by the rat. Fd. Chem. Toxic., Vol 23, No. 11, pp. 1001 – 1009, 1985 (TOX2002-831)
Guidelines:	Not stated
Deviations:	The test design is insufficient. Only one treated group was used. The feed used for treatment was stated to have contained 5 ppb phosphine (but without analytical proof). By using standard conversion factors, this would correspond to a dose level of only about 0.0025 - 0.005 mg/kg bw/d, which is far below the lowest dose in the inhalation study. Furthermore, presentation and discussion of results (especially of pathomorphology) is insufficient.
GLP:	Not stated
Acceptability:	The study is considered to be not acceptable.

WARNING: This document forms part of an EC evaluation package and should not be used in isolation. Registration must be started before use of this document.

Materials and methods:

Phosphine released from aluminium phosphide, batch no: not stated; purity: not stated. Dietary toxicity study carried out over a period of 2 years on 120 weanling Sprague-Dawley rats (60 male and 60 females). The control group (30 males and 30 females) received a standard laboratory diet and the treated group (30 males and 30 females) received the same diet previously fumigated with phosphine (2000 ppm in the sealed containers with bulk diet). The resulting average residual level of phosphine in diet was stated to be 5 ppb. Observations and examinations: mortality, general appearance behaviour, body weights, food consumption, haematology, clinical chemistry, urinalysis, organ weights, macroscopic and histopathological examinations; Sacrifice after one year, 19 male and 20 female control animals and 20 male and 19 female test animals, and all surviving after two years of treatment.

Findings:

No relevant differences on general condition and behaviour of animals were observed.

No significant difference persisted in body weights, and the body weight gain was very similar in rats of control and treated groups. The laboratory investigation showed no difference of biological significance between control and treated groups. Interim sacrifice after one year: Relative organ weights did not show significant differences between control and treated groups with the exception of increased thymus weights in females. Terminal sacrifice after two years: Relative thymus weight of treated female animals was increased and lung weight of treated females was decreased. The presentation and discussion of results of pathomorphology in the submitted paper is inconsistent and inadequate.

Conclusion:

The authors of this study stated no substance related effects. However, test design and presentation and discussion of the results are insufficient. Therefore, the study is considered to be not acceptable.

B.6.5.3 Carcinogenicity study – mouse

A carcinogenicity study with magnesium or aluminium phosphide in the mouse has not been conducted. Based on lack of exposure and the absence of genotoxic concern waiving of a long term/carcinogenicity study in a second species was seen as justified.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

An acceptable two generation reproduction study was not submitted. However, long-term exposure is negligible and there is a very steep dose response curve of metal phosphide / phosphine toxicity from which it can be expected that maternal toxicity would dominate over reproductive effects. Therefore, a waiving concept was accepted. A 2-generation oral study in rats with fumigated diet (fumigation with phosphine) was published. No effects have been observed, however, the study was considered to be not acceptable. The effects of phosphine gas on pregnancy/embryo-foetal development were evaluated in a developmental toxicity study in the rat (summary see Table B.6.6-1). Treatment was by inhalation with phosphine, the gas produced by aluminium phosphide. Phosphine administered by whole body inhalation to pregnant females rats at target exposure levels up to 5.0 ppm for 6 hrs/day over the day 6 - 15 of gestation interval was not maternally toxic, embryotoxic, foetotoxic or teratogenic. However at the 7.5 ppm exposure, the first 14 mated females on test died during the day 8 - 15

gestation interval after receiving three to 10 days of exposure. Therefore, the No Observed Effect Level (NOEL) for the maternal and developmental toxicity for this study in rat was 5 ppm. The analytical concentration of this target dose was 4.9 ppm, equivalent to 0.007 mg/L air or 1.9 mg/kg bw/day.

Table B.6.6-1: Summary of maternal and developmental toxicity

Study and dose levels (mg/kg/day)	NO(A)EL	LOEL	Reference
Rat 2-generation study with fumigated diet	No effects in result of fumigation. Concentration of as in diet not measured. The study is not acceptable	No effects in result of fumigation. Concentration of as in diet not measured.	Cabrol, 1986 (TOX2005-189)
Rat developmental toxicity 0, 0.03, 0.3, 3.0, 5.0 and 7.5 ppm (by inhalation)	Maternal toxicity: NOEL: 5 ppm	Maternal toxicity: LOEL: 7.5 ppm Based on mortality	Schroeder, 1989 (TOX2001-687)
	Developmental toxicity: NOEL: 5 ppm *) Equivalent to 0.007 mg/L air or 1.9 mg/kg bw/day	Developmental toxicity: LOEL: > 5 ppm Up to 5 ppm no developmental tox. was observed, dose group 7.5 ppm was early terminated	

*) = The analytical concentration was 4.9 ppm.

B.6.6.1 Reproduction study in the rat with phosphine fumigated diet

Report: Cabrol, A.-M. et al. (1986): Reproduction in the rat with phosphine fumigated diet, published in: Microbiol. Aliments Nutrition, Vol. 4, pp. 241-246, 1986 (TOX2005-189)

Guidelines: Not stated

Deviations: No guideline study. The concentration of the test substance in the diet was not measured.

GLP: No

Acceptability: The study is considered to be not acceptable.

Material and methods:

Phosphine, concentration during fumigation of diet: 200 ppm; animals: Sprague-Dawley rats, number of rats: 10 female, 4 male, age: 1 to 7 months (generations F₀, F₁ and F₂). Body weight at start of treatment (generation F₀): females: 106 ± 8 g; males: 106 ± 8 g acclimatisation: seven days under laboratory conditions after veterinary examination. Conditions: air conditioned with adequate fresh air supply, temperature 23 ± 1 °C, relative humidity 50 ± 10 % and 12 hours light and 12 hours darkness, solid diet: 12 % water, 20 % protein, 54.5 % glucoside, 4 % cellulose, 5.5 % minerals. Observations and examinations: fertility index, viability index, nutritional index and biological or clinical values in plasma and urine; statistics: students` t test.

Findings:

No substance related effects have been observed. However, the design of the study was insufficient. Therefore, the study is considered to be not acceptable. Results are summarised in Table B.6.6-2, Table B.6.6-3, and Table B.6.6-4, respectively.

Table B.6.6-2: Parameters of reproduction in two generations of rats (n = 10) fed by fumigated diet (results obtained are in percentage)

	F ₀ F ₁	F ₀ F ₁	F ₁ F ₂	F ₁ F ₂
	Control	Treated	Control	Treated
Fertility index	70	90	100	100
Gravidity index	100	100	100	100
Viability index	67 ± 13.1	71 ± 14.4	83 ± 10.3	75 ± 11.4
Nutritional index	100 ± 0	95 ± 4.7	80 ± 13.5	89 ± 8.9

Table B.6.6-3: Haematological analyses evaluated in two generations of rats fed by fumigated diet (age of rats: 2 months, n = 6)

	Generation F ₁				Generation F ₂			
	Male		Female		Male		Female	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Number of erythrocytes x 10 ⁶ /mm ³	7.9 ± 2.4	8.5 ± 4.5	7.9 ± 1.5	7.3 ± 4.6	7.0 ± 0.5	6.8 ± 0.2	6.7 ± 0.4	6.6 ± 0.4
Number of leucocytes x 10 ³ /mm ³	15.8 ± 1.1	13.2 ± 0.9	16.1 ± 1.8	13.6 ± 2.2	9.6 ± 0.7	7.8 ± 1.5	9.1 ± 1.0	6.5 ± 0.7

Table B.6.6-4: Urine analyses in two generations of rats fed by fumigated diet (age of rats = 2 months, observation time = 24 hours, n = 6)

	Generation F ₁				Generation F ₂			
	Male		Female		Male		Female	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Na ⁺ mg/ 24 h	53 ± 4.6	49 ± 9.4	36 ± 5.1	44 ± 1.6	37 ± 6.3	55 ± 6.9	34 ± 3.1	34 ± 7.5
K ⁺ mg/ 24 h	123 ± 21.8	126 ± 17.2	87 ± 11.6	127 ± 18.3	105 ± 24.0	149 ± 14.4	98 ± 7.7	88 ± 15.4
Phosphorus total mg/ 24 h	31 ± 4.1	20 ± 3.2	21 ± 3.6	15 ± 1.8	24 ± 4.2	27 ± 4.5	22 ± 3.7	18 ± 2.9
Urea g/ 24 h	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
Creatinin mg/ 24 h	14.9 ± 1.0	15.6 ± 2.1	9.8 ± 1.1	11.5 ± 0.4	18.9 ± 4.2	18.8 ± 1.0	13.7 ± 0.9	13.2 ± 2.6
Diuresis mL/ 24 h	15 ± 0.8	13 ± 1.6	15 ± 2.5	14 ± 1.5	15 ± 1.0	18 ± 1.8	18 ± 1.1	17 ± 2.6
pH	6.8 ± 0.2	7.1 ± 0.3	7.5 ± 0.5	7.1 ± 0.2	7.0 ± 0.3	8.1 ± 0.2	7.5 ± 0.2	8.0 ± 0.3

Conclusions:

Under the conditions of work, in animals fed by fumigated diet, no effects of the active substance has been observed. However, the design of the study was insufficient. Therefore, the study is considered to be not acceptable.

B.6.6.2 Teratogenicity by the inhalation route – rat

Report:	R. E. Schroeder (1989): An Inhalation Developmental Toxicity Study of Phosphine (PH ₃) in Rats, Bio/dynamics Inc., East Millstone, USA, unpublished report no. 89-3413, 05.12.89 (TOX2001-687).
Guidelines:	United States Environmental Protection Agency Pesticide Assessment Guideline (Subdivision F: Hazard Evaluation; Human and Domestic Animals; Office of Pesticide Programs); Section 83-3 Teratogenicity Study (November 1984).
Deviations:	No
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test material: Phosphine, batch numbers N-429433, H-70377, N-299593, N-411001, gaseous, 1 % phosphine/nitrogen.

Phosphine was administered by whole-body inhalation into the breathing zone as vapour to mated CD rats (24 mated females/group) from days 6 – 15 of gestation. Target exposure levels were 0.03, 0.3, 3.0, 5.0 and 7.5 ppm. In this study a sham-air, chamber-exposed control group was included.

Study animals were observed twice daily for mortality/morbidity and for obvious pharmacologic and/or toxicologic effects and each female was given a detailed physical examination on days 0, 6 – 15 and 20 of gestation. Females were weighed on days 0, 6, 10, 12, 16 and 20 of gestation and food consumption data were recorded for days 0 – 6, 6 – 10, 10 – 16 and 16 – 20 of gestation. All surviving females were sacrificed on day 20 of gestation and given a gross postmortem evaluation. The uterus of each female was removed, weighed intact and evaluated for the number of fetuses and resorption sites. The ovaries were also dissected free and the number of corpora lutea recorded. Fetuses recovered at this time were evaluated for external malformations/variations, sexed and weighed.

Subsequently, one-half of the fetuses in each litter were processed for visceral evaluation (microdissection procedure) and the remaining fetuses were processed for staining of the ossified structures with Alizarin Red S and evaluated for skeletal malformations and/or ossification variations.

Findings:

The overall mean analytical concentrations were close to target and nominal values were 0.0, 0.034, 0.33, 2.8, 4.9 and 7.0 ppm. Temperatures and relative humidity values within the chambers during exposure intervals were considered acceptable. There was no significant gradient of test material concentration within the chambers as evident from pre-test chamber distribution evaluations.

Only at the 7.5 ppm exposure level an adverse effect of treatment was evident from maternal mortality. No mortality occurred in the control group or in groups treated at the 0.03 ppm, 0.30 ppm, 3.00 ppm or 5.00 ppm exposure levels. All females in these groups (groups I-V) survived to scheduled sacrifice. In group VI (7.5 ppm) the first 14 females on test died during the exposure period (days 8 - 15 of gestation) after three to 10 days of exposures. Due to this mortality, the 7.5 ppm exposure group was terminated early.

No adverse effect of treatment was evident from the detailed physical evaluation of females in the control or treated groups. Even at the 7.5 ppm exposure level which was terminated due to increased mortality, no adverse effect of treatment was evident from physical observation data.

No adverse effect of treatment up to an exposure level of 5.0 ppm was evident from bodyweight, bodyweight change data and food consumption data.

No adverse effect of treatment to an exposure level of 5.0 ppm was evident from the gross postmortem examination data. Reddening of the lungs and liver was observed in some of the group VI (7.5 ppm) animals that died during the study but these findings were not considered unusual in animals, which die and are not exsanguinated prior to postmortem examination.

Pregnancy rates for groups 0-0.03-0.3-3.0-5.0 ppm were 91.7 %, 87.5 %, 100.0 %, 95.8 % and 95.8 %, respectively, therefore no adverse effect of treatment up to the 5.0 ppm exposure level was evident from pregnancy rates (see Table B.6.6-5). A total of seven females in these groups were not pregnant.

The mean number of corpora lutea, uterine implantation sites and viable foetuses per pregnant female and the mean pre-implantation loss indices were comparable between the control and treated groups 0.03 - 5.0 ppm.

The mean numbers of resorption sites per pregnant female, the mean resorption/implant ratio and the incidence of females with resorptions among their uterine implants was comparable between the control and treated groups 0.3-5.0 ppm. In the low exposure group (group II, 0.03 ppm) the mean number of resorption sites, the mean resorption/implantation ratio and the incidence of females with resorptions were higher than control and these differences were statistically significant. These same data for the low dose group were also outside the range of recent historical control data for this laboratory. The increase in resorption data for this low-exposure group (group II) was not attributable to an increase in resorption data for a single litter. In the absence of a similar response at the higher exposure levels (0.3 ppm, 3.0 ppm and 5.0 ppm) this change was not considered to be treatment related.

Thus, to an exposure level of 5.0 ppm, no adverse effect of treatment was evident from uterine implantation data.

No adverse effect of treatment was evident from foetal weight, sex distribution or external variation in morphology.

The dissimilar malformations seen among the treated groups occurred at low incidence and their occurrence in this study was not considered indicative of a treatment related effect.

No adverse effect of treatment at up to 5.0 ppm was evident from visceral and skeletal evaluations of fetuses.

Table B.6.6-5: Summary of reproduction data from an inhalation developmental toxicity study of phosphine in rats

	Dose groups				
	0 ppm	0.03 ppm	0.3 ppm	3.0 ppm	5.0 ppm
Females mated	24	24	24	24	24
Pregnant (%)	22 (91.7)	21 (87.5)	24 (100)	23 (95.8)	23 (95.8)
Corpora Lutea	16.0 ± 2.0	16.3 ± 1.9	16.0 ± 2.0	15.9 ± 2.5	16.1 ± 2.3
Implantation sites	15.4 ± 2.0	15.7 ± 1.8	14.7 ± 2.4	15.0 ± 2.8	15.4 ± 2.3
Mean preimplantation loss	0.039	0.036	0.079	0.059	0.039
Mean litter size	14.9	14.1	14.0	14.2	14.7
Resorptions (mean)	12 (0.5)	33 (1.6)**	17 (0.7)	20 (0.9)	17 (0.7)
Mean resorptions/implants	0.036	0.098*	0.045	0.056	0.051
Litter with resorptions (%)	8 (36.4)	16 (76.2)*	9 (37.5)	14 (60.9)	10 (43.5)
Foetal weight (g)	3.24 ± 0.4	3.18 ± 0.25	3.26 ± 0.17	3.28 ± 0.23	3.22 ± 0.20

*= p ≤ 0.05 (Fisher exact test)

**= p ≤ 0.01 (Fisher exact test)

Conclusions:

Phosphine administered by whole body inhalation to pregnant females CD[®] rats at target exposure levels of 0.03 ppm, 0.3 ppm, 3.0 ppm and 5.0 ppm for 6 hrs/day over the day 6 - 15 of gestation interval was not maternally toxic, embryotoxic, foetotoxic or teratogenic. At the 7.5 ppm exposure level, the first 14 mated females on test died during the day 8 - 15 of gestation interval after receiving three to 10 days of exposure. Therefore, this dose group was terminated early. The NOEL for maternal toxicity and developmental toxicity in rat was 5 ppm (the analytical concentration of this nominal dose was 4.9 ppm).

B.6.6.3 Teratogenicity study – rabbit

A teratogenicity study in rabbits was not submitted. The justification for non-submission is based on negligible long-term exposure and the steep dose response curve of metal phosphide toxicity from which it can be expected that maternal toxicity would dominate over reproductive effects. Phosphine was not embryotoxic, foetotoxic or teratogenic in the rat study. A waiving is considered to be acceptable.

B.6.7 Delayed neurotoxicity (Annex IIA 5.7)

The neurotoxicity of phosphine has been assessed in rats in an acute and a 90-day inhalation study (see Table B.6.7-1).

In the acute neurotoxicity study, rats were exposed to 0, 20, 30 and 40 ppm phosphine gas (nominal conc.) administered via whole body inhalation exposure for one session of four hours duration. The No Observable Adverse Effect Level (NOAEL) of phosphine in rats was 40 ppm (analytical conc. 38 ppm) with regard to anatomic pathology and the behavioural and neurological status observed in the functional observational battery, and less than 20 ppm with regard to changes in motor activity on day 1.

In the subchronic neurotoxicity study, rats were exposed to phosphine gas via whole body exposure at levels of 0.3, 1 and 3 ppm, 6 hours per day, 5 days per week, for 13 weeks. Due to equivocal effects seen in high dose males, and the lack of effects seen in females the No Observed Adverse Effect Level (NOAEL) of phosphine for systemic/neurotoxic effects in rats exposed over a 90-day period is 3 ppm, the highest dose tested in this study.

Table B.6.7-1: Overview of neurotoxicity studies on phosphine

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value NOAEL	Reference
Rat acute neurotoxicity Whole body inhalation, 4 h US EPA Series 81, 82, 83, 82-4	Rats, Sprague Dawley albino 11M+11F	Phosphine 0, 20, 30, 40 ppm (nominal conc.) (analytical conc.: 0, 21.2, 27.6, and 38.4 ppm)	Motor activity: NOAEL < 21 ppm FOB and histopathology NOAEL = 38 ppm	Schaefer, 1998 (TOX2000-97)
Rat repeat-dose neurotoxicity whole body inhalation, 6h/day, 5d/week, 13 weeks EU 2004/73/EC, B.43 (2004)	Rats, Charles River CrI:CD BR VAF/Plus 16M+16F, recovery 6M+6F	Phosphine 0, 0.3, 1, 3 ppm	Neurotoxic/ systemic NOAEL 3 ppm	Schaefer, 1998 (TOX2006-176)

B.6.7.1 Acute neurotoxicity – rat

Report: Schaefer, G.J. (1996): Acute neurotoxicity study in rats, 750-002, 1996 Phosphine task force, MPI Research, Mattawan, MI 49071, USA, 31 July 1998 (TOX2000-97)

Report: Schaefer, G.J. et al. (1998): Acute and subchronic inhalation neurotoxicity of phosphine in the rat, Inhalation Toxicol. 10, pp. 293-320, 1998 (TOX2002-190)

Deviations: Food consumption was not measured. Batch not mentioned.

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and Methods:

Groups of 11 male and 11 female albino Sprague-Dawley albino rats (CrI: CD BR VAF/Plus, provided by Charles River Laboratories, Michigan 49081, USA) were exposed to 0, 20, 30 and 40 ppm phosphine gas administered to rats via whole body inhalation exposure for one session of four hours duration. Another group of 11 males and 11 females served as the negative control and was exposed to clean air.

One percent phosphine gas in nitrogen (99 %) was diluted with the chamber ventilation air to produce the desired exposure atmosphere. The nominal concentration (ppm) was determined by multiplying the total volume (litres) of test material delivered to the chamber by the concentration of the test material (10.000 ppm phosphine) and dividing by the total volume of air passed through the exposure chamber. The amount of test material used during the exposure was calculated by multiplying the test material flow rate by the duration of exposure. Actual chamber concentrations were determined four times each hour by delivering chamber atmosphere samples to a gas chromatograph (GC) positioned beside the chamber for analysis.

The animals underwent a detailed clinical examination once each week and were observed twice daily for mortality, morbidity and injury during the 14 day post-exposure period.

Body weights were recorded just prior to the exposure and at 7 and 14 days post-exposure.

Functional observational batteries (FOB) consisting of quantitative and qualitative neurobehavioral parameters (observations in the home cage, during handling and in the openfield, and sensimotor evaluations) were performed on each animal pre-test, at the estimated time of peak effect within 8 hours post-exposure and at 7 and 14 days post-exposure. Motor activity parameters were assessed for each animal on a comparable regimen using a Digiscan[®] Activity Monitor for three consecutive 10-minute intervals (30 minutes total).

Following the in-life neurotoxicity evaluation, six rats per sex per group were randomly selected for neuropathology evaluation; a thorough post mortem examination was conducted on the remaining animals. Absolute organ weights from all animals were recorded along with post mortem body weights and appropriate weight ratios (relative to body and brain weights) were calculated.

Chamber airflow, temperature, and humidity were monitored continuously throughout the exposure and recorded at 30-minute intervals. The oxygen content of the chamber atmosphere was measured once during the exposure.

The main exposure parameters were as follows, see Table B.6.7-2.

Table B.6.7-2: Main exposure parameters

Exposure period	4 hours
Exposure chamber volume	16.000 L
Chamber air flow	3500 L/minute
Mean chamber temperature	23 – 24 °C
Mean relative humidity range	47 – 62 %
Target concentration	0, 20, 30 and 40 ppm
Nominal concentration	0, 20, 30 and 40 ppm
Mean analysed concentration	0, 21, 28 and 38 ppm

Findings:

All animals survived to study termination. No significant clinical signs were noted during the study, although a single 40 ppm group animal did exhibit emaciation. No remarkable changes in the body weights of animals were noted during the study with the exception of the animal exhibiting emaciation. Although some statistical significance was noted in body weights, it was not considered article related and all the other animals appeared to gain weight normally during the study.

No significant instances of behavioural toxicity were noted in the functional observational battery examinations. The differences observed were considered random and therefore not dose related.

The motor activity evaluations exhibited a consistent pattern characterised by a reduction in all four activity parameters in both sexes on day 1 for the 20, 30 and 40 ppm groups when compared with animals in the 0 ppm group (see Table B.6.7-3). These decreases were particularly evident during the 10 and 20 minute intervals. Reduction in activity parameters was also observed during the 30 minute interval, but these changes were not significant. While these changes appear to be test article-related, they no longer occurred at 7 and 14 days after dosing.

Table B.6.7-3: Summary of motor activity of rats on day 1 after exposure to phosphine (group mean values for 10-minute intervals)

Time interval (minutes)	Males				Females			
	0 ppm	20 ppm	30 ppm	40 ppm	0 ppm	20 ppm	30 ppm	40 ppm
Horizontal activity (count)								
0 - 10	3188	751**	901**	518**	4217	1212**	2191**	694**
10 - 20	1073	240**	123**	22**	1552	220*	391	161*
20 - 30	621	66	115	58	259	119	91	80
Vertical activity (count)								
0 - 10	1019	412**	256**	343**	850	497**	406**	247**
10 - 20	484	74**	14**	26**	351	121	45 ¹	31*
20 - 30	209	29	8	38	51	37	1	23
Total distance (cm)								
0 - 10	2066	417**	586**	309**	2906	573**	1434**	410**
10 - 20	679	66*	36*	4*	945	71	144	32
20 - 30	406	21	25	23	96	32	6	24
Stereotypic time (sec)								
0 - 10	104	43**	33**	26**	126	65**	75**	31**
10 - 20	34	11**	6**	1**	65	12*	21	9*
20 - 30	24	2	7	2	17	7	6	5

* Significantly different from the control group: $p \leq 0.05$ ** Significantly different from the control group: $p \leq 0.01$

There were no definitive macroscopic test article related effects or test article related organ weight changes observed. Observations noted were considered to be incidental or unrelated to test article administration. Similarly, no test article related findings were observed in the neuropathology segment.

Conclusion:

NOAEL (No Observable Adverse Effect Level): 38 ppm (with regard to anatomic pathology and the behavioural and neurological status observed in the functional observational battery) and < 20 ppm (with regard to changes in motor activity on day 1).

B.6.7.2 Delayed neurotoxicity following acute exposure – hen

The chemical structures of zinc phosphide/phosphine have no relationship with compounds known to induce delayed neurotoxicity, e.g. organophosphorus compounds that can cause neuropathy target esterase inhibition. Consequently, no such study has been conducted.

B.6.7.3 28-day delayed neurotoxicity – hen

The chemical structures of zinc phosphide/phosphine have no relationship with compounds known to induce delayed neurotoxicity, e.g. organophosphorus compounds that can cause neuropathy target esterase inhibition. Consequently, no such study has been conducted.

B.6.7.4 Subchronic 90-day neurotoxicity – rat

Report:	Schaefer, G.J. (1998, TOX): A 90-day inhalation neurotoxicity study of phosphine in rats, 750-003, 1996 Phosphine task force, MPI Research, Mattawan, MI 49071, USA, 31 July 1998 (TOX2006-176)
Guidelines:	EU 2004/73/EC, B.43 (2004)
Deviations:	Food consumption was not measured.
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Materials and Methods:

Hydrogen phosphide, phosphine, PH₃, lot/batch no.: 1A017105, 1A4424, 1A9257, 1A9299, 1A016812, 1C1098, 1C1882, and 1A020359; purity: 1 % phosphine electronic grade, 99 % nitrogen electronic grade.

Three groups (groups 2, 3 and 4) each consisting of 16 male and 16 female Charles River CD[®] albino rats were exposed to phosphine administered as a gas via whole body exposure at levels of 0.3, 1 and 3 ppm, 6 hours per day, 5 days per week, for 13 weeks. Another group of 16 males and 16 females served as the negative control and was exposed to clean air (group 1). Additional 6 males and 6 females were assigned to groups 1 and 4 and were used for a 2 week recovery group. In each group, 11 animals per sex were designated as behaviour animals, and 6 of these were designated for neuropathological examinations. All animals not assigned to neuropathology constituted the toxicity phase of the study. One percent phosphine gas in nitrogen (99 %) was diluted with the chamber ventilation air to produce the desired exposure atmosphere.

The nominal concentration (ppm) was determined by multiplying the total volume of test article delivered to the chamber by the concentration of the test article. This product was divided by the total volume of air passing through the exposure chamber to give the nominal concentration. The amount of test article used during the exposure was calculated by multiplying the test article flowrate by the duration of exposure. Actual chamber concentrations were determined each hour during the 6-hour exposure by delivering chamber atmosphere samples to a gas chromatograph (GC).

All animals were observed for mortality, morbidity and injury twice daily, 7 days a week throughout the study. The rats were observed twice daily for signs of toxicity at the times of the mortality/morbidity checks. Detailed clinical examinations were conducted once each week. Individual body weights were obtained pre-test and weekly throughout the study.

After at least 65 days of exposure (and a 2-week recovery period for the recovery group animals), various haematologic, serum biochemical, urinalysis, necropsy and histopathologic evaluations were conducted on samples collected from the 11 males and 11 females from each group not designated for neuropathology. An ophthalmoscopic examination was conducted on each rat once during the pre-test period and at study termination (week 13).

Functional observational batteries (FOB) consisting of quantitative and qualitative neurobehavioural parameters (observations in the home cage, during handling and in the openfield, and sensorimotor evaluations) were performed in all groups at pre-test, and during weeks 4, 8 and 13 of exposure, as well as after a 2-week recovery period for the recovery group animals. Following all other FOB measurements, individual animal body weight and body temperatures (measured rectally) were recorded. Motor activity parameters were assessed for each animal on a regimen comparable to and following the FOB regimen. Each animal was monitored in a Digiscan[®] Activity Monitor for 3 consecutive, 10-minute intervals (30 minutes total time).

Following the in-life neurotoxicity evaluation, 6 animals/sex/group were randomly selected for neuropathology evaluation from the 11 animals/sex/group designated as behaviour test animals. Animals in the recovery study were also prepared for neuropathology evaluations; a thorough postmortem examination was conducted on the remaining animals.

For main exposure parameters see Table B.6.7-4.

Table B.6.7-4: Main exposure parameters

Exposure period	6 hours/day
Exposure chamber volume	16.000 L
Chamber air flow	3500 L/minute
Mean chamber temperature	22.5 ± 1.7 °C
Relative humidity range	55.8 ± 9.8 %
Target concentration	0, 0.3, 1 and 3 ppm
Nominal concentration	0, 0.25, 0.97 and 3.02 ppm
Actual concentration	0, 0.30, 1.01 and 2.99 ppm

Findings:

The nominal concentrations were 0.25, 0.97 and 3.02 ppm of phosphine respectively, while the analytically determined actual concentrations were 0.30, 1.01, and 2.99 ppm.

Three animals died during the course of the study. A male of dose group 0.25 ppm died on study day 101 and a female of group 3 ppm died on study day 89, but the cause of death could not be determined. In addition, a male of group 3 ppm died on study day 102 after blood collection.

There were no significant clinical findings observed during the study period

Body weights were slightly higher in high dose (males 2.4 %, females 1.2 %) after 13 weeks of treatment and became equal or less than the control body weights after the 2 week recovery period.

There were no treatment related findings in the haematological and serum biochemical or urinalysis parameters evaluated.

Palpebral closure was dose related increased in males, and reached statistic significance in high dose males at week 4. Body temperatures was dose related decreased in males and reached statistical significance at week 13. However, the overall evaluation of the functional observational battery assessment was that the test article did not produce consistent or enduring effect in the behavioural or neurological status of the animals.

The horizontal and vertical motor activities were significantly lower in high dose males at week 13, and were consistently, but not significant lower at other time intervals. Motor activity measurements in females were compromised by high variations and significant decreases in the high dose group at the pretest interval.

There were no treatment related findings at necropsy or at neurohistopathological examination of collected tissues.

There were no definitive test article-related organ weight changes observed.

Conclusion:

Due to equivocal effects seen in high dose males, and the lack of effects seen in females the No Observed Adverse Effect Level (NOAEL) of phosphine for systemic/neurotoxic effects in rats exposed over a 90-day period is 3 ppm, the highest dose tested in this study.

B.6.7.5 Postnatal developmental neurotoxicity – rat

No evidence of a neurotoxic potential was seen in the rat acute and subchronic (90-day) toxicity studies. Therefore, it is considered unnecessary to conduct a postnatal developmental toxicity study.

B.6.8 Further toxicological studies (Annex IIA 5.8)

It was demonstrated that phosphine or other phosphide derived reaction products induced Heinz body formation in relatively low concentrations (1.25 ppm) in normal human erythrocytes. The time course for the induction of Heinz bodies is relatively slow (4 h). The formation of Heinz bodies by phosphine is oxygen-dependent, consistent with earlier work regarding the insecticidal properties of the chemical. Finally, these *in vitro* data lead to the speculation that prolonged *in vivo* exposure to phosphine in concentrations exceeding the PEL might have an adverse effect on haemoglobin in susceptible segments of the worker population exposed to the chemical.

The results of another study show that after acute poisoning of rats by phosphine the respiration of the isolated liver mitochondria is diminished. The oxidation of α -ketoglutarat turned out to be the most sensitive. The oxidative phosphorylation, however, remains on a normal level. In general, the disturbance equals that of phosphine action on isolated mitochondria *in vitro*. Similar effects have been observed on the isolated sarcosomes of heart muscle of poisoned animals on an early state of intoxication. But in the sarcosome respiration and phosphorylation is uncoupled at the same time. Since the respiration of *Neurospora crassa* is also decreased by phosphine it is to assume that this agent acts by this mechanism on living cells in general. The same kind of disturbance can be demonstrated in the mitochondria after chronic administration of doses which are far below the toxic ones of phosphine and by which animals do not show any sign of damage. There is a small but considerable fall of CoA in the liver of acute poisoned animals.

Report:	Potter, W.T. et al. (1991): Phosphine-mediated Heinz body formation and hemoglobin oxidation in human erythrocytes, published in: Toxicology Letters, 57, pp. 37-45, 1991 (TOX2000-113)
Guidelines:	No guideline study
Deviations:	Not applicable
GLP:	No
Acceptability:	The study is considered to be supplementary.

Material and methods:

Phosphine generated from magnesium phosphide pellets; stock phosphine solutions were prepared by adding 1.0 mL of 70 % ethanol to 4.0 ± 0.2 mg of magnesium phosphide in gas-tight vials (total volume of 15 cc). Heparinised blood was obtained from healthy donors by venipuncture and used within 2 days. For *in vitro* assessments of red-cell Heinz body formation, 1.0 mL of whole blood was mixed with 14 mL of Hanks' balanced salt medium supplemented with 25.0 mM Hepes buffer (pH 7.1) in air-tight-Teflon-sealed vials with a total volume of 25 cc. Phosphine dose response curves were performed on individual blood samples with injections of either the stock phosphine/ethanol solution (0-100 μ L) or head space gas. Treated and control samples were incubated at 37 °C for time periods up to 24 h.

Red cells suspension was then mixed and stained for Heinz bodies with a freshly prepared, filtered 0.5 % methyl violet. Cytospin slides were prepared after 5 min and the number of cells containing Heinz bodies were counted from 10 fields of 100 cells each. Erythrocytes were prepared for electron microscopy by fixation in 0.5 v/v 3.0 % glutaraldehyde in White's buffered saline (pH 6.8) for 30 min at 4 °C. The cells were sedimented by low-speed centrifugation and refixed in 3.0 % glutaraldehyde in White's saline for 1 h at 4 °C. Cells were then post-fixed with 1.0 % osmium tetroxide, 1.5 % ferrocyanide for 1 h at 4 °C. The cells were dehydrated through an ascending series of ethanol (50, 70, 90, 100 %) for 15 min at each step and embedded in Spurr (EM Sciences). Thin sections (60 nm) were collected on copper grids and stained with uranyl acetate and lead citrate. Sections were examined in a Philips 300 electron microscope at an accelerating voltage of 80 kV. Haemoglobin was obtained from human packed red cells previously washed 3 times in isotonic saline prior to hypotonic lysis using a 5-fold excess of pure water for 30 min at 20 °C. Incompletely lysed cells and membrane stromal material were removed by centrifugation. The resultant Hb solution was dialysed for 24 h against 100 mM KCl, 25 mM NaPO₄ buffer (pH 7.2) with 3 changes of buffer. Formation of the deoxygenated or carbonyl derivatives was accomplished by blowing purified nitrogen or CO over gently stirred haemoglobin solutions. Gas exchange was monitored using a Cary 119 UV/visible spectrophotometer thermostatted at 20 °C. The identification and monitoring of haemoglobin reaction products was made spectrophotometrically using previously published spectral extinction coefficients of Antonini and Brunori. Phosphine reactions on oxygenated, deoxygenated and carbonyl haemoglobin derivatives were performed by direct injection of either phosphine/70 % ethanol stock solutions or the head space gas directly into silicon rubber septum-sealed 1-cm path-length cuvettes. Oxidation kinetics were monitored by the measuring of absorbance at time intervals. Analytic apparatus: *In vitro* assessment of red cell Heinz body formation was monitored by means electron microscopy (Philips 300 electron microscope) to explore the mechanisms of phosphine intoxication. The identification and monitoring of haemoglobin reaction products was made spectrophotometrically (Cary 119 UV/visible spectrophotometer) using previously published spectral extinction coefficients of E. Antonini and M. Brunori (Hemoglobin and Myoglobin in their Reactions with Ligands, Frontiers of Biology, Vol. 21, North-Holland Publishing Co., New York, 1971).

Findings:

Phosphine-induced Heinz bodies in human erythrocytes: The exposure of human erythrocytes to phosphine results in a dose-dependent development of Heinz bodies. Phosphine concentrations greater than 1.25 ppm were required for observable Heinz bodies. The extent of Heinz body formation after 4 h of phosphine exposure was only slightly less (~ 5 %) than that observed following a 24-h exposure; however, the development at exposure times of less than 4 h was more variable. In general, Heinz bodies were not observed following only 1 h of incubation at the 2.0 ppm levels or lower. The majority of the erythrocytes contained more than one Heinz body per cell at phosphine concentration of 3 ppm or greater. The lesions were similar in appearance the those formed with much higher concentrations of phenylhydrazine. The average size and shape was highly variable, suggesting multiple nucleation sites. Most Heinz bodies exhibited an irregular contour and many, but not all, were associated with slight protrusions or invaginations of the cell membrane. At the 3 ppm levels of phosphine exposure, some haemolysis was observed (up to ~ 20 %). Pretreatment of the erythrocytes by flushing with carbon monoxide for 20 min eliminated the formation of Heinz bodies. Under these conditions residual dioxygen is present, but at low levels. Phosphine-induced haemoglobin oxidation and degradation The publication contains figures with typical spectroscopic changes induced in oxyhaemoglobin by addition of 0.1 µM phosphine. Over the initial 8 h of the

reaction time course the spectra indicate a slow conversion of HbO₂ to metHb (isosebestic points at 590 and 524 nm). The pseudo-first-order rate constant obtained indicates that the initial reaction occurs without a lag phase. After approximately this first third of the total reaction course, absorbances at longer wavelengths increase, isosebestic points shift and multiple unresolved spectral species are indicated. Absorbances at wavelengths up to 700 nm increase with the accompanied protein precipitate formation. The final spectrum is not indicative of pure metHb state but most closely approximates that of haemichrome. The oxygen dependence of the phosphine-mediated degradation of Hb was investigated using HbCO and deoxyHb. Both HbCO and deoxyHb showed no evidence of metHb formation even with prolonged exposure to 0.4 µM phosphine.

Conclusions:

The present work demonstrates for the first time that phosphine or other phosphide derived reaction products induced Heinz body formation in relatively low concentrations (1.25 ppm) in normal human erythrocytes. The time course for the induction of Heinz bodies is relatively slow (4 h). The formation of Heinz bodies by phosphine is oxygen-dependent, consistent with earlier work regarding the insecticidal properties of the chemical. Finally, these *in vitro* data lead to the speculation that prolonged *in vivo* exposure to phosphine in concentrations exceeding the PEL might have an adverse effect on haemoglobin in susceptible segments of the worker population exposed to the chemical.

Report:	Neubert, D. and Hoffmeister, I. (1960): Veränderungen im intermediären Stoffwechsel nach der Einwirkung von Phosphorwasserstoff, Naunyn-Schmiedeberg's Arch. exp. Path. u. Pharmak. 239, pp. 219-233, 1960 (TOX96-52055)
Guidelines:	No guideline study
Deviations:	Not applicable
GLP:	No
Acceptability:	The study is considered to be supplementary.

Material and methods:

Phosphine, in predetermined dosages, generated in the glass cages by reaction of crystalline phosphonium iodide with 0.1 N solution of potassium hydroxide. Animals: Wistar-rats, female (staff E 3). Acclimatisation: The rats were given standard food and water ad libitum up to the beginning of the tests. Dosage of phosphine: 0.1 to 4.64 mg/L. Housing: 121 L cages of glass, good sealed, air conditioned with adequate fresh air supply. Test with microorganisms: The microbiological studies were performed with *Neurospora crassa* wildtyp 7a.

After acute poisoning by phosphine *in vivo* and *in vitro* investigation of the respiration and the oxidative phosphorylation in the respiration chain; tests with liver cells (liver-mitochondria); tests with isolated sarcosomes of heartmuscle tests with microorganisms of type *Neurospora crassa*. After chronic poisoning investigation of the respiration and the oxidative phosphorylation in the respiration chain. After poisoning determination of the concentration of CoA in the liver and the activity of ferments as acetylating agents.

Findings:

Results are summarised in Table B.6.8-1, Table B.6.8-2 and Table B.6.8-3:

Table B.6.8-1: Acute poisoning by phosphine. Calculation of concentration x time product ($c \cdot t$) (mg/L x min) from the mid-point of range of survival times.

Concentration of phosphine c (mg/L)	Lethal time t (min)	$c \cdot t$ (mg/L \cdot min)
4.64	19	87
3.16	-	-
2.15	31	67
1.47	39	57
1.00	54	54
0.681	70	48
0.464	110	51
0.316	158	50
0.215	220	47
0.147	-	-
0.100	490	49

Table B.6.8-2: Toxicity of phosphine by multiple dosages

Concentration (mg/L)	Lethal time acute (min)	Number of animals	Chronic poisoning		
			Day 1 (min)	Day 2 (min)	Died
0.68	65-75	8	46	25	8
1.47	35-50	10	25	25	8

Table B.6.8-3: Influence on respiration and the oxidative phosphorylation in the respiration chain by phosphine. Tests with liver cells (liver- mitochondria) *in vivo*. Phosphine dosage: 0.68 mg/L. Test time: 40 - 45 min, Substrate: α -ketoglutarat. The animal no. 8 was in a lethargic stage.

Test	Animal no.	QO2	μ AO	μ MP	P/O
Check-up	1	152	4.0	11.9	3.0
	2	195	6.4	21.4	3.3
	3	240	4.4	12.9	2.9
	4	171	6.3	17.2	2.7
	5	263	5.0	15.6	3.1
	6	207	7.3	21.7	3.0
Phosphine dosage	7	51	1.3	3.4	2.6
	8	26	1.0	0	0
	9	70	1.8	4.9	2.7

Conclusion:

After acute poisoning of rats by phosphine the respiration of the isolated liver mitochondria is diminished. The oxidation of α -ketoglutarat turned out to be the most sensitive. The oxidative phosphorylation, however, remains on a normal level. Similar effects have been observed on isolated mitochondria *in vitro* and on the isolated sarcosomes of heartmuscle of poisoned animals on an early state of intoxication. But in the sarcosome respiration and phosphorylation

is uncoupled at the same time. Since the respiration of *Neurospora crassa* is also decreased by phosphine it is to assume that this agent acts by this mechanism on living cells in general. The same kind of disturbance can be demonstrated in the mitochondria after chronic administration of doses which are far below the toxic ones of phosphine and by which animals do not show any sign of damage. There is a small but considerable fall of CoA in the liver of acute poisoned animals.

B.6.9 Medical data and information (Annex IIA 5.9)

Among the examined persons, occupied in the production of Polytanol (Calcium phosphide), no health impairment was detected over a period of 3 to 16 years. The case reports are considered to be representative of the numerous records of poisoning cases, mainly in connection with suicide, which are available from the literature. Diagnosis is mainly based on the history of intake, gastrointestinal symptoms, shock symptoms and silver nitrate impregnated paper test. Main symptoms are severe circulatory, cardiac, and renal failure, uraemia, hepatic damage, changes in ECG, and respiratory distress connected with a high mortality rate. Histopathological changes have mainly been observed in lungs, liver, heart and kidney. Since an antidote is not available, therapy relies on treatment of the clinical symptoms and administration of high doses of corticoids.

B.6.9.1 Report on medical surveillance on manufacturing plant personnel

Report:	Konerding, J.(2004): Arbeitsmedizinische Bescheinigung; Chemische Fabrik Wülfel GmbH & Co. KG, Hannover, Germany, unpublished report, April 05, 2004 (TOX2004-882) and Köhler, U., Arbeitsmedizinische Vorsorgeuntersuchungen des Betriebspersonals- Direkte Beobachtungen; Chemische Fabrik Wülfel GmbH & Co. KG, Hannover, Germany, unpublished report, March 15, 2004 (TOX2005-194)
Guidelines:	No guideline study
Deviations:	Not applicable
GLP:	No
Acceptability:	The report is considered to be acceptable.

Material and methods:

Statement on medical surveillance on manufacturing plant personnel by Dr. med. Konerding (Occupational physician, Hannover, Germany): All workers involved with the production of calcium phosphide are regularly monitored at intervals of 12 months by occupational medical inspections according to the requirements of the German national regulations on hazardous substances (Gefahrstoffverordnung). In addition to this, special comprehensive health check ups are conducted in intervals of 36 months according to recommendation no. 26 (relevant where protective respiratory devices are used) of the professional trade association (Berufsgenossenschaft). In the regular health inspections, the following parameters are assessed: differential blood count, blood glucose, creatinine, liver status parameters, haematocrit, urine sampling. Furthermore, technical medical check ups are carried out like hearing tests, vision tests, lung function tests, x-ray investigations of the chest, and ECG and tests on olfactory nerve.

Findings:

Nine persons in total were examined from 1987 to 2003. The examinations are performed annually. The examinations according to G25 (driving, steering and supervision abilities) and according to G26.2 (medium respiratory protection, full mask) occur every three years. The different time periods for the examined persons are the result of the duration of the respective occupational circumstances. Among the examined persons, occupied in the production of Polytanol (Calcium phosphide), no health impairment was detected over a period of 3 to 16 years.

Conclusion:

Among the examined persons, occupied in the production of Polytanol (Calcium phosphide), no health impairment was detected over a period of 3 to 16 years.

Report:

Garry, V.F. et al.(1989): Human Genotoxicity: Pesticide Applicators and Phosphine, published in: Science, 246, pp. 251-255, 1989 (TOX2006-28) (TOX2000-114)

Guidelines:

No guideline study

Deviations:

Not applicable

GLP:

No

Acceptability:

The study is considered to be acceptable.

Material and methods:

Phosphine was generated from magnesium phosphide. Standard culture and harvest techniques were used. A mixture of RPMI 1640 (Gibco), 20 % fetal bovine serum (Hyclone lot no. 1115583), and phytohemagglutinin (PHA) (Gibco) (0.75 µg/mL) was routinely used as the culture medium for all cytogenetic studies. The same lot of fetal bovine serum was used throughout the study. Cells were harvested after 48 hours of culture for nonbanded chromosome analysis and stained with 2 % Giemsa. Portions (100 µL) of phosphine dissolved in solvent were withdrawn through a Teflon faced septum and added to cell cultures. Within one half hour of addition to culture, phosphine was extracted from the culture with toluene and processed for gas chromatography. The gas chromatographic method used is a modification of A. Vinsjansen and K. E. Thrane (Analyst 103, 1195 (1978)). Fumigant workers who, 6 weeks to 3 month earlier, were exposed to phosphine, a common grain fumigant, or to phosphine and other pesticides had significantly increased stable chromosome rearrangements, primarily translocations in G banded lymphocytes. Less stable aberrations including chromatid deletions and gaps were significantly increased only during the application season, but not at this later time point. During fumigant application, measured exposure to phosphine exceeds accepted national standards. This refers in particular to several *in vitro* cytogenicity tests with inhalation exposure to phosphine, and to a gene mutation test (HPRT mutation assay in mice spleen cells) involving exposure of the test of fumigant workers over a period of 6 weeks to 3 month 0, 0.26, 1.4 and 4.5 ppm of phosphine. To index and characterise phosphine exposure, personal breathing zone measurements were made on members of the study group who allowed the access to fumigant application. Among workers involved in enclosed space application (grain bin), exposures ranged from 0.4 to 5.8 mg/m³ (n=10) with a mean of 2.97 mg/m³. Phosphine release from the phosphide occurred in some instances in as little as 5 min. Among workers involved in open air application (rail car), exposure ranged from 0.1 to 0.90 mg/m³ (n=4). Analytical methods: Sister chromatid exchange analysis was performed on 72 hour cultures to which bromodeoxyuridine (BrU) (8µg/mL) was added at 24 hours. Cells were stained by the Hoechst + Giemsa method. All

samples were coded and scored. Phosphine analysis was performed according to National Institute for Occupational Safety and Health (NIOSH) method S-332. Phosphine standards were prepared from zinc phosphide (D. Hill, in Analytic Methods for Pesticide and Growth Regulators, G. Zweig and J. Sherma, Eds., Academic Press, New York, 1986, p. 145).

Findings:

Chromosome aberrations were compared among different applicator groups, grain workers, and control subjects (see Table B.6.9-1). The data from each applicator, consisting of samples studied two or more times during the fumigant application season, were pooled and expressed as the average rate per 100 cells for chromosome aberrations. In each sample, 100 cells were counted per exposed subject. Grain workers and controls were similarly studied over the fumigant application season. The combined data for each subject was converted to a square root scale to homogenise the intersubject variation within groups. Statistical significance (t test after significant F) was determined by analysis of variance on the converted scale. In these analyses, exposure related trends, differences among group means, and possible smoking related influences were considered. Mean \pm SE and range for the untransformed data are presented. Gaps are defined as achromatic regions within a chromatid less than the width of the chromatid. Deletions in the nonbanded preparations are nonstaining regions in a chromatid greater than the width of the chromatid. Breaks are a discontinuity in a chromatid or chromosome that is misaligned. Rings, dicentrics, quadriradial figures, and acentric fragments result from exchanges within or between chromatids. Applicators as a group demonstrate significantly increased aberrations compared to grain workers or control subjects with gaps excluded in the computation. Applicators who use phosphine alone compared to applicators who used other pesticides and fumigants but not phosphine and compared to control subjects have significantly increased gaps and deletions.

Table B.6.9-1: Results of chromosome aberrations among different applicator groups

Mitotic cells counted	Gaps	Deletions	Breaks	Rings, dicentrics, quadriradial figures, acentric fragments	Total (excludes gaps)
Phosphine alone (n = 9)					
2400	5.92 \pm 1.00 2.5 to 12.3	2.52 \pm 0.53 0 to 4.7	1.64 \pm 0.28 0 to 3	0.46 \pm 0.28 0 to 2	4.62 \pm 0.74 1.5 to 8.5
Phosphine and other pesticides (n = 11)					
3600	2.86 \pm 0.54 0 to 6	1.45 \pm 0.48 0 to 4.7	1.67 \pm 0.34 0 to 4	0.55 \pm 0.16 0 to 1.5	3.67 \pm 0.79 1 to 9
Other pesticides and fumigants (n = 4)					
800	1.25 \pm 0.52 0 to 2.5	1.62 \pm 1.01 0 to 4.5	1.25 \pm 0.32 0.5 to 2	0.88 \pm 0.24 0.5 to 1.5	3.75 \pm 0.83 1.5 to 5.5
State grain workers (n = 15)					
1500	2.33 \pm 0.51 0 to 6	1.20 \pm 0.24 0 to 6	0.87 \pm 0.32 0 to 4	0.07 \pm 0.07 0 to 1	2.14 \pm 0.65 0 to 9
Control (n = 24)					
2400	3.3 \pm 0.51 0 to 8	0.54 \pm 0.20 0 to 3	0.71 \pm 0.21 0 to 4	0.13 \pm 0.09 0 to 2	1.38 \pm 0.31 0 to 5

G₀-stage human lymphocytes in complete medium were exposed to phosphine for 20 min at room temperature in gas-tight vials, aerated for one-half hour, and cultured for 96 hours with added PHA. The nonbanded chromosome aberration frequencies for each phosphine dose are the data from five separate studies. At least 125 mitotic cells per dose were counted. The aberration rate per 100 cells is shown in Table B.6.9-2 shown. There is a significant ($P < 0.01$) dose related increase for gaps, deletions, and total aberrations excluding gaps (linear regression analysis).

Table B.6.9-2: Chromosome aberration frequencies induced by different doses of phosphine added in vitro.

Dose (µg/L)	Mitotic cells Counted	Gaps	Deletions	Breaks	Rings, dicentrics, acentric fragments	Total (excludes gaps)
Control	200	3.5	0.05	0.1	0	0.15
ND*	125	4.0	3.0	2.0	0	5.0
0.26	125	6.4	6.4	0.8	2.0	9.2
1.40	125	7.2	5.6	7.2	1.6	14.4
4.50	125	8.8	10.4	4.0	1.6	16.0

*ND, not detectable by gas chromatography

Report: Sorensen, K.J. (1996): The effect of phosphine, smoking and age on stable chromosome aberration frequencies in agricultural workers. Environ. Mol. Mutagen. 27, Suppl. 27, 64. (TOX2006-29)

Guidelines: No guideline study

Deviations: Not applicable

GLP: No

Acceptability: The study is considered to be supplementary.

Material and methods:

Whole chromosome painting probes were used to determine whether occupational exposure to phosphine leads to detectable cytogenetic damage in agricultural workers. Blood samples were collected from 50 individuals (23 workers occupationally exposed to phosphine and 27 non exposed subjects) in Minnesota aged 18 – 62 including smokers and non-smokers. Chromosome 1, 2 and 4 were painted simultaneously and 1000 cell equivalents were analysed from each donor to determine the frequency of stable aberrations and aneuploidy.

Findings:

No differences in the frequency of stable chromosome aberrations or aneuploidy were found in the phosphine exposed group or the smoking group. A significant increase in the frequency of stable aberrations with age was found.

Conclusion:

The lack of detail on the extent and nature of exposure does not allow any precise allocation of potential effects of exposure to phosphine.

Report:	Garry, V.F. et al. (1992): Chromosome rearrangements in fumigant applicators: possible relationship to non-Hodgkin's lymphoma risk. Cancer Epidemiology 1, 287-291. (TOX2006-30)
Guidelines:	No guideline study
Deviations:	Not applicable
GLP:	No
Acceptability:	The study is considered to be supplementary.

Material and methods:

Study investigating chromosomal aberrations in professional fumigant applicators. The following study groups were included: (1) 6 fumigant applicators using phosphine almost exclusively during the application season (5 of these workers discontinued the use of phosphine during the 2-year study period), (2) 12 workers who applied herbicides and insecticides in their primary work and used phosphine for occasional fumigation work, (3) 26 control subjects. Blood specimens were taken approximately one year apart from each of the 18 applicators. One hundred G-banded metaphase cells from each subject were analysed for chromosome aberrations.

Findings

A significant increase in chromosome re-arrangements in G-banded chromosomes from peripheral blood was observed in applicators of pesticides (n = 18) who were exposed to the fumigant phosphine or who had mixed exposure to other pesticides and phosphine when compared to a control group. Applicators who had discontinued the use of phosphine for at least 8 month prior to specimen collection (n = 5) did not demonstrate significant increases in chromosome rearrangements (see Table B.6.9-3).

Table B.6.9-3: Chromosome breakage and re-arrangements in G-banded metaphase cells (expressed as rate/100 metaphase cells)

Group	No. of subjects	No. of mitoses	Breaks	Rearrangements
Phosphine	6	700	4.1 ± 1.0	1.7 ± 0.5
Ceased phosphine	5 ^a	500	3.0 ± 1.0	0.0
Mixed exposure	12	2205	3.5 ± 0.6	1.4 ± 0.4
Controls	26	2533	3.3 ± 0.4	0.5 ± 0.1

^a repeat measurements performed in 2 of 5 subjects six month after cessation of phosphine use are not included in the table The average frequency of breaks was 5 % and average frequency of rearrangements was 1.0 % with 100 metaphases examined from each of these two subjects.

Breakpoint analysis indicated four bands (1p13, 2p23, 14q32, 21q12) with a significant excess of breaks in the exposed group and no breaks in the control group. Most breaks at bands 1p13, 14q32 and 21q22 were associated with chromosome rearrangements and occurred in applicators who have a mixed exposure to phosphine and other pesticides. Cytogenetic abnormalities, i.e. rearrangements and/or deletions involving bands 1p13, 2p13 and 14q32 are associated with non-Hodgkin's lymphoma.

Conclusion:

The lack of detail on the extent and nature of exposure (indoors or outdoors as well as the combination with other pesticides) does not allow any precise allocation of the claimed effects to the action of phosphine.

Report: Amr, M. et al. (1993): Neurobehavioural changes among workers in some chemical industries in Egypt; Environ. Res. 63, 295-300 (TOX2002-201)

Guidelines: No guideline study

Deviations: Not applicable

GLP: No

Acceptability: The study is considered to be supplementary.

Material and methods:

Test group (see Table B.6.9-4): 46 industrial workers occupationally exposed to zinc phosphide in Egypt; Control group: non-exposed subjects; Recordings were made of: (1) detailed history: personal, occupational, present and past medical histories, (2) neurological and psychiatric assessment, (3) fundus examination, (4) laboratory tests: EEG and in some cases EMG (electromyography).

Table B.6.9-4: Study population exposed to zinc phosphide

Parameters	Zinc phosphide
Number	46
Age (years)	28-46
Mean	35.8
Duration of exposure (years)	5-21
Mean	11.3

Findings:

EEG tracings were abnormal in 17.4 % of subjects exposed to zinc phosphide, and in EMG studies, partial denervation of anterior tibial and flexor digiti minimi muscles in 2/30 workers exposed to zinc phosphide were noted. A high level of fear of poisoning was striking in zinc phosphide exposed workers, speculated upon by the authors occupational exposure poses a heavy psychological stress and is associated with signs of anxiety. For further details on neuropsychiatric symptoms related to zinc phosphide exposure please refer to Table B.6.9-5:

Table B.6.9-5: Frequency distribution of neuropsychiatric symptoms and signs among the studied population

Parameters		Zinc phosphide n = 46) frequency (%)	Non-exposed (n = 300) frequency (%)
Symptoms	Headache	24.0	8.0
	Impotence	15.0	3.0
	Numbness and parasthesia	2.2	11.0
	Anxiety and irritability	19.6	10.0
	Muscle weakness and easily fatigued	9.0	3.0
	Tremors	0.0	2.0
	fear of poisoning	87.0	0.0
	Other symptoms	9.0	6.0
	Symptom free	13.0	80.0
signs	Abnormal reflexes	23.9	4.0
	Hyposthesia (peripheral)	19.6	7.0
	Mask faces	0.0	0.0
	Muscle weakness and static tremors	6.5	2.0
	Fundus changes	0.0	6.0

Parameters		Zinc phosphide n = 46) frequency (%)	Non-exposed (n = 300) frequency (%)
	Psychiatric changes	13.0	12.0
	Constricted pupil	0.0	0.0
	EEG change	17.4	1.0
	Other	13.0	1.0
	sign free	54.4	76.0

Conclusion:

In the opinion of the authors, exposure to zinc phosphide of industrial workers raises a number of concerns over possible neuropsychiatric symptoms and other signs such as headache, anxiety and irritability, abnormal reflexes, hyposthesia and others. However, for lack of job descriptions and process information, it can not be excluded that zinc phosphide is not the only agent to which these workers were exposed, which is why the interpretation of this report should be with care.

Report:

Amr, M. et al. (1997): Neuropsychiatric syndromes and occupational exposure to Zinc phosphide in Egypt, Environmental Research 73, 200-206 (TOX2006-27)

Guidelines:

No guideline study

Deviations:

Not applicable

GLP:

No

Acceptability:

The study is considered to be supplementary.

Material and methods:

Test group (see Table B.6.9-6): 86 industrial workers occupationally exposed to zinc phosphide in Egypt; control group: non-exposed subjects; recordings were made of: (1) detailed history: personal, occupational, present and past medical histories, (2) neurological and psychiatric assessment, (3) EEG recording in 46 subjects, EMG studies were conducted in 30 members of the exposed group who had neuromuscular symptoms or signs, (4) assessment of clinical chemistry parameters for liver and kidney function (including GPT, GOT, LDH, and urea), (5) determination of serum levels of Zn, Cu, Fe, sodium, potassium, Ca, P, Mg, total lipids, phospholipids, total cholesterol, triglycerides and amino acids, (6) ECGs and postero-anterior view x-rays of the chest.

Table B.6.9-6: Study population exposed to zinc phosphide

Parameters	Zinc phosphide
Number	86
Age (years)	28-46
Mean	35.8
Duration of exposure (years)	5-21
Mean	11.3

Findings:

Serum zinc and calcium levels were significantly increased while Cu, Fe, P and Mg levels were significantly decreased in exposed workers compared to the control group (Table B.6.9-7). The electrophoretic pattern of globulin showed that the gamma-globulin fraction was significantly increased while α_2 and β - globulin levels were decreased (Table B.6.9-9). The lipoprotein pattern showed that total lipids, B-lipoprotein and B/ α ratio were significantly

increased in exposed workers and α_1 lipoprotein was decreased (Table B.6.9-8). Triglycerides and cholesterol levels and several hepatorenal function parameters (i.e. glucose, urea, GPT, GOT, AP, LDH, γ -GT) were significantly increased in the exposed group (Table B.6.9-10). Phospholipids and phospholipids/cholesterol ratio were significantly decreased. A total of 68.6 % of the exposed workers had chest symptoms, only 24.4 % presented with chest or cardiac signs. Respiratory functions were abnormal in 70 % of the exposed workers and abnormal ECG findings were present in 12.8 %.

Table B.6.9-7: Mean values of serum levels of zinc and other elements

Group	Zn (µg/dL)	Cu (µg/dL)	Fe (µg/dL)	Ca (mg %)	P (mg %)	Mg (mg %)	Na (mEq/L)	K (mEq/L)
Control (n = 20)	131.50	113.8	126.70	8.85	5.05	2.25	141.1	4.5
Exposed (n = 30)	215.9	70.1	77.9	10.14	3.75	1.76	142.7	4.66
p value	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.025	ns	ns

* ns: not significant

Table B.6.9-8: Mean values of serum lipids and their subfractions

	Parameter (mg %)								
Group	Total lipids	Tri-glyce-rides	Phospho-lipids	Cholest-erol	Phospholipid / cholesterol (%)	Lipoprotein pattern			
						α	β	non mobile	β/α
Control (n = 20)	560	175.5	222.2	162	0.37	192.5	300.9	72.1	1.56
Exposed (n = 32)	628	223.2	195.16	213.13	0.92	160.2	367.3	104	2.34
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table B.6.9-9: Mean values of serum proteins and electrophoretic pattern (g/100 mL)

Group	Total proteins	Albumin	Globulins					Albumin/ globulin ratio
			α_1	α_2	β	γ	Total G	
Control (n = 20)	6.93	3.74	0.35	0.78	0.93	1.13	3.19	1.16
Exposed (n = 25)	6.47	3.64	0.27	0.42	0.55	1.54	2.85	1.28
p value	ns	ns	ns	< 0.001	< 0.001	< 0.001	< 0.01	ns

* ns: not significant

Table B.6.9-10: Hepatorenal function

Group	Glucose (mg/dL)	Urea (mg/dL)	GPT (u/mL)	GOT (u/mL)	Alk. ph. (KU/dL)	LDH (u/mL)	γ -GT (IU/L)	T. proteins (g/dL)	Globulin (g/dL)
Control	77.1	28.3	19.8	21.2	5.13	179.3	24.7	7.36	2.7
Exposed	103.9	33.27	31.22	26.54	9.21	605.52	53.52	7.91	3.35
p value	< 0.001	< 0.005	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01

Conclusion:

In the opinion of the authors, exposure to zinc phosphide of industrial workers raises a number of concerns over possible neuropsychiatric symptoms and other signs such as headache, anxiety and irritability. In addition, they concluded that the results of the study indicated that exposure to zinc phosphide caused mild acute and sub-acute liver cell damage and affected renal function and perhaps B-cells of the pancreas. However, (i) for lack of job descriptions and process information, and (ii) for lack of any exposure data related to zinc / zinc phosphide, it can neither be excluded that zinc phosphide is the only chemical to which these workers were exposed, nor can zinc quantitatively be identified as a causative agent. Thus, the reliability of the information given in this publication should be considered as very limited.

Report: Tucker, J.D. et al. (2003): Multi-endpoint biological monitoring of phosphine workers; Mutation Research 536, 7-14 (TOX2005-336)

Guidelines: No guideline study

Deviations: Not applicable

GLP: No

Acceptability: The study is considered to be supplementary.

Material and methods:

Test group: 22 industrial workers occupationally exposed to phosphine divided into smokers and non-smokers. Control groups: 26 non-exposed subjects (smokers and non-smokers); two independent methods were used to evaluate exposure: (1) fluorescence in situ hybridisation (FISH) with whole-chromosome paints of chromosomes 1, 2 and 4 labelled in a single colour to quantify translocations in peripheral lymphocytes, (2) glycophorin A (GPA) assay to quantify phenotypically mutant erythrocytes.

Findings:

No effect of phosphine applicator status or cigarette smoking on translocation frequencies was observed (Table B.6.9-11) and no significant difference was observed among any of the three sub groups of phosphine applicators (Table B.6.9-12). The results of multiple linear regression analysis showed a significant increase in the frequency of translocations with age, but there are no effects of phosphine applicator status or cigarette smoking.

Table B.6.9-11: Donor age, chromosome translocation frequencies and GPA variant frequencies in the study population by applicator status and cigarette smoking

	Control		Phosphine applicators	
	non-smokers	ever-smokers	non-smokers	ever-smokers
Age (Mean \pm SE)	36.0 \pm 2.4	41.9 \pm 2.7	37.0 \pm 2.1	43.1 \pm 3.9
All translocations				
No. of subjects evaluated	11	15	9	11
Mean per 100 cell equivalents \pm SE	0.370 \pm 0.054	0.511 \pm 0.064	0.275 \pm 0.028	0.390 \pm 0.038
GPA				
No. of subjects evaluated	3	7	3	3
NN frequency per million cells \pm SE	10.0 \pm 1.8	11.5 \pm 2.4	6.2 \pm 0.6	10.0 \pm 2.9
NØ frequency per million cells \pm SE	5.4 \pm 0.9	5.5 \pm 1.1	2.0 \pm 0.4	4.6 \pm 1.5

Table B.6.9-12: Donor age and chromosome translocation frequencies in the applicator sub-populations

	Auto-dispensers	Other uses	No current use
Age (Mean \pm SE)	47.0 \pm 4.9	37.1 \pm 2.5	39.3 \pm 5.0
Total translocations			
No. of subjects evaluated	5	8	7
Mean per 100 cell equivalents \pm SE	0.491 \pm 0.136	0.301 \pm 0.053	0.272 \pm 0.089

Conclusions

No differences in the frequency of translocations were found in the phosphine applicators compared to the controls, and no effect of cigarette smoking was observed. A significant increase in the frequency of translocations with age was seen in all groups. No effect of phosphine exposure or cigarette smoking was observed in the GPA assay. In the opinion of the authors, the results of this study can be interpreted as supporting the effectiveness of the personal protective equipment that is now worn by the workers but which was not employed prior to and during the earlier studies, which showed an increase in chromosome aberrations among phosphine applicators.

Further reports:

Barbosa and Bonin (1994, TOX97-50675): The authors examined a small cohort of 31 fumigators who had worked with phosphine for a mean of 11.6 years (range between 1.5 and 32 years). Phosphine concentration in the breathing zone of fumigators was recorded during eight fumigations, with the highest level recorded being 2.4 ppm/hour, although more typical concentrations were < 0.1 ppm/hour. These workers and 21 controls matched by sex, age, and smoking habit had haematological profiles, whole serum and blood cholinesterase activities, and several clinical biochemistry measures monitored. The results for micronuclei showed no significant differences between fumigators and controls, but detected a strong association between age and increased frequency of micronuclei. Measurement of urine mutagenicity did not show any significant difference between fumigators and controls, but did show increased excretion of mutagens in smokers. No significant effects were seen in any parameter monitored, including genotoxic endpoints. At monitored occupational exposures of < 2.4 ppm/h the results revealed no association between phosphine exposure and genotoxic or toxicological effects in fumigators.

Guth (2003, TOX2004-2205): In this study all workers involved in the production of aluminium phosphide containing products were regularly monitored by IAS at intervals of 12 months since 1988 and at intervals of 24 months since 2000. In this health inspection the following parameters are assessed: physical examination, sight and hearing test if required, urine examination (no further specification), differential blood count, transaminase and cholinesterase activity and, in various cases, examination of breathing equipment, driving, steering, and overseeing work, and mercury (concentration in blood? - no further description given). It was concluded that the health examinations conducted with the plant personnel showed no negative health effects during the investigation period of ten years.

Misra et al. (1988, TOX2006-182) studied 22 fumigation workers in India. These workers placed aluminium phosphide pellets in grain piles which were then covered air-tight for a period of 1 week. The mean age of the workers was 48 years (range 24 – 60) and mean duration of exposure 11.1 years (range 0.5 – 29) and workplace concentrations of phosphine

gas ranged from 0.17 to 2.11 ppm during the period of investigation. After fumigation they reported symptoms, which included cough (18.2 %), dyspnoea (31.8 %), tightness around the chest (27.3 %), headache (31.8 %), giddiness, numbness and lethargy (13.6 % each), anorexia and epigastric pain (18.2 % each). The abnormal physical signs included bilateral diffuse rhonchi and absent ankle reflex each occurring in one worker. Motor nerve conduction velocity of median and peroneal nerves, and sensory conduction velocity of median and sural nerves were normal.

Asher (2005, TOX2006-225): In the years of 1997, 2000 and 2002a, total of 15 factory workers in the manufacturing plant of aluminium phosphide have had regular health check. According to the author, there was no relation of workers' health status to exposure to metal phosphide and phosphine gas.

Environmental Protection Agency (1999): In an Australian study of workers exposed to phosphine, 31 phosphine fumigators and 21 controls, all employed at the New South Wales Grain Corporation, were examined for micronucleus incidence in peripheral blood lymphocytes and their concentrated urine was assessed for mutagenicity in TA100 and TA98 strains of *S. typhimurium*. In addition, serum bile acids were measured. The subjects, all males, were matched for medication, X-ray exposure within the past year and smoking habits. There was no indication how often the fumigators were exposed, or the most recent exposure date or the length of the various fumigators employed. No individual data were presented to identify if certain individuals showed unusually high micronuclei incidence or presence of mutagens in the urine. Urine samples were concentrated 75-fold and the procedure of Yamaski and Ames (1977) was used to test mutagenicity to TA100 and TA98 in the presence or absence of metabolic activation (S9). There was no increase in the mutagenicity of urine from the fumigators (N=27) vs controls (N=19) in this assay. Serum bile acids showed no changes related to phosphine exposure. Cholesterol and some liver enzymes (gamma-glutamyl transferase) were elevated in the exposed group. Micronuclei formation was measured in isolated peripheral blood lymphocytes cultured for 44 hours in the presence of phytohemagglutinin to stimulate mitosis, arrested at metaphase with cytochalasin-B and harvested by cytocentrifugation after 72 hours in culture. The micronucleus incidence was comparable among the fumigators and the control groups (overall MI for fumigators = 6.9 vs 7.1 for controls).

B.6.9.2 Report on clinical cases and poisoning incidents

Inquiries were made to several German institutions dealing with intoxication incidents, specifically addressing cases of Zinc phosphide poisoning:

Brockstedt (1991, TOX2006-688): Inquiry with regard to the active substance zinc phosphide - Engl. transl. of German doc.: Anfragen zum Wirkstoff Zinkphosphid; Written communications, Beratungsstelle für Vergiftungserscheinungen, Berlin, Germany.

Martens (1991, TOX2006-692): Inquiry with regard to the pesticide active substance - Engl. transl. of German doc.: Anfrage zu Pflanzenschutzmittelwirkstoff Zinkphosphid, Giftinformationszentrum, Universitätsklinikum Virchow, Berlin, Germany.

Kowaleswski; Smend (1991, TOX2006-25): Inquiry with regard to the pesticide active substance - Engl. transl. of German doc.: Anfrage zu Pflanzenschutzmittelwirkstoff Zinkphosphid, Informationszentrale gegen Vergiftungen, Friedrich-Wilhelms-Universität Bonn, Bonn, Germany.

Gellner (1991, TOX2006-696): Inquiry with regard to the pesticide active substance - Engl. transl. of German doc.: Anfrage zu Pflanzenschutzmittelwirkstoff Zinkphosphid, Medizinische Klinik, Wilhelms-Universität Münster, Münster, Germany.

Moll (1991, TOX2006-697): Inquiry with regard to the pesticide active substance - Engl. transl. of German doc.: Anfrage zu Pflanzenschutzmittelwirkstoff Zinkphosphid, Marienhospital, Papenburg, Germany.

The response to inquiries on poisoning incidents involving zinc phosphide can be summarised as follows (Table B.6.9-13):

Table B.6.9-13: Request on zinc phosphide poisoning cases

Reference	Institute/Hospital	No. of all incidents	Zinc phosphide related incidents
5.9.2/01 Brockstedt (1991) (TOX2006-688)	Bezirksamt Charlottenburg von Berlin, Beratungsstelle für Vergiftungserscheinungen und Embryonaltoxikologie	in 1990: 49.591 reported incidents (total)	total reported cases: 74 attempted suicide: 13 accidents involving children: 61
5.9.2/02 Martens, F. (1991) (TOX2006-692)	Freie Universität Berlin, Universitätsklinikum Rudolf Virchow, Reanimationszentrum/ Giftinformationszentrum	1975-1991: a total of 40 zinc phosphide related cases	total reported cases: 40 adults: 37 children: 1 household pets: 2
5.9.2/03 Kowaleswski, S.; Smend, J. (1991) (TOX2006-25)	Informationszentrale gegen Vergiftungen, Rhein. Friedr.-Wilhelms-Universität Bonn	1986-1991: a total of 11 zinc phosphide cases reported	total reported cases: 11 human exposures: 9 household pets: 2
5.9.2/04 Gellner, R. (1991) (TOX2006-696)	Westfälische Wilhelms-Universität Münster, Medizinische Klinik u. Poliklinik	1990-1991	no incidents with plant protection products containing with zinc phosphide were reported
5.9.2/05 Moll H. (1991) (TOX2006-697)	Marienhospital, Pädiatrische Abteilung	1990-1991	Only one case of planned (not performed) suicide with zinc phosphide reported

Further reports:

The literature concerning phosphine exposure in humans consists of studies in worker populations exposed by inhalation and reports of high dose exposure by the oral route. Phosphine is usually ingested as a metal phosphide (e.g. aluminum phosphide). The acidic environment of the stomach catalyses the hydrolysis of the metal phosphide releasing phosphine gas (Siwach et al., 1988, TOX2006-191). There is a wide range of symptoms resulting from this exposure, including nausea and vomiting, restlessness, metabolic acidosis, hypotension, shock, sweating, tachycardia, oliguria, tachypnea, and dyspnea (Chugh et al., 1989, TOX2007-168; Gupta et al., 1995, TOX2006-188; Khosla et al. 1988, TOX2007-170; Siwach et al., 1988, TOX2006191). There are some reports of cardiac dysfunction (Gupta et al., 1995 TOX2006-188, Khosla et al., 1988, TOX2007-170) and adrenocortical toxicity (Chugh et al., 1989, TOX2007-168). Ingestion also involves a high mortality (25-70 %). Ingestion of one or more aluminum phosphide fumigation tablets produces 1 gram of phosphine (Chugh et al., 1991, TOX2005-305).

Ninety-two patients with aluminium phosphide poisoning due to ingestion were studied over a period of 3 years. Abdominal pain, vomiting and restlessness were the common initial features followed by alteration in sensorium and shock unresponsive to conventional treatment. Electrocardiographic abnormalities were very common and highly variable. Routine serum

biochemistry was usually unremarkable. Severe metabolic acidosis was common and mortality high (49 %). The survivors recover completely without any residual organ damage (**Khosla et al., 1992, TOX2006-189**).

Harger and Spolyar (1958, TOX2007-171); Wilson et al. (1980, TOX2007-172): There have been a few deaths attributed to high levels of inhaled phosphine gas. The signs and symptoms following exposures are very similar to those observed following oral exposure. There are several descriptions of acute toxicity following inhalation exposure to high concentrations of phosphine in the literature. However, in many of these cases toxicity appeared to be reversible. **Glass (1956, TOX2006-187)** reported phosphine poisoning of 11 members of a submarine crew. The men became dizzy and short of breath, developed visual disturbances, and vomited; some complained of palpitations and chest tightness, and seven required hospitalisation. Preliminary examinations revealed weak and rapid pulses, dyspnoea, and moist sounds at the base of the lungs. One man had a drop in blood pressure to 100/50 that returned to normal by the next day. By day 3, all men had recovered fully and were returned to active duty.

Similar symptoms were reported in 29 of 31 crew members and 2 children aboard a freighter which was loaded with phosphine-fumigated grain (**Wilson et al., 1980, TOX2007-172**). Exposure concentrations were not reported. Laboratory findings revealed occult blood (n = 5), bile (n = 3), and glucose (n = 1) in the urine. Liver function abnormalities were noted: serum glutamic pyruvic transaminase (SGPT) (n = 5), α -glutamyl transpeptidase (GGTP) (n = 1), and lactate dehydrogenase (LDH) (n = 5) were increased, and cholinesterase was decreased in one individual. Four of the crew and both children required hospitalisation. Two of the four crew members had elevated creatinine phosphokinase (CPK) values, one had tachycardia, and two had decreased sensation to pinprick in their lower extremities. One child died and the other child had tachycardia with ST segment depression and CPK elevation. All clinical and laboratory findings on the survivors were normal after 6 days of hospitalisation.

Three federal grain inspectors were reported to be overcome by phosphine gas after opening a railcar containing phosphine-fumigated grain (**Feldstein et al., 1991, TOX2006-186**). The workers immediately closed the railcar and 1 hour later reported symptoms at a local emergency room. None required hospitalisation and one worker was asymptomatic 4 days later. Another worker was still symptomatic 4 days later; however, the clinical evaluation and complete blood count were normal. Three weeks later the patient still complained of shortness of breath, while the other symptoms had resolved. It normalised after one week and the patient remained asymptomatic. The third worker reported episodes of disorientation and daily occipital headaches. However, EEG and neurological exams were normal. The phosphine level of the railcar was 2.029 ppm 20 minutes after aeration.

The findings of inhalation studies described above indicate that acute phosphine toxicity can result from exposure to very high short-term exposures. However, the effects are reversible among survivors with clinical and laboratory findings returning to normal in a few days.

Michael O'Malley of the California Department of Pesticide Regulation has examined past exposures to the rodenticide fumigant aluminium phosphide. Between 1982 and 1992 a total of 29,863 pesticide incidents occurred in California, 181 were associated with aluminium phosphide or its phosphine by-product. There was one fatality involving an unemployed man who stowed away in a rice filled railcar undergoing fumigation, who was found dead several days later when the train reached its destination. In another case, a person fumigating silos at a wheat milling operation without respiratory protection, passed out while driving home from work. Cases associated with improper disposal of unspent residues accounted for 31 (15 %) of the aluminium phosphide cases.

Zipf et al. (1967, TOX2005-287): In this detailed case report of aluminium phosphide poisoning a 25-year-old gardener's labourer swallowed 6 Phostoxin tablets (70 % aluminium phosphide and approximately 30 % ammonium carbamate) dissolved in water as suicide attempt. The poison was inhaled as well since the tablets have been dissolved in water which causes release of phosphine gas. In the hospital, an extensive gastric lavage with potassium permanganate and magnesium sulphate was carried out. The following findings were noted: The patient had experienced severe pain behind the sternum and in the epigastric region accompanied by an unbearable feeling of heat and burning throughout the body after swallowing the poison. Severe circulatory, cardiac and renal failure and liver damage resulted. Clearly apparent changes in ECG and EEG were found. The histological findings for liver and kidneys corresponded to a great extent with those stated in the literature, thus providing intra vitam confirmation. One probable reason for the man having survived drinking a lethal dose of Phostoxin is that he immediately vomited the major portion of the poison. A further reason is that, due to the characteristic carbide odour, the nature of the poisoning could be recognised immediately and appropriate treatment commenced without delay. The application of extracorporeal haemodialysis and medication with heart and circulatory preparations contributed decisively towards prevention of a lethal course.

NIOSH Alert document (Anonymous, 1999, TOX2006-226): Californian authorities reported typical cases of poisoning incidents in relation with metal phosphide and phosphine gas.

Burgess et al. (1998, TOX2002-203): Fumigant related illness: Washington State's five year experience, published in: J. Toxicol. Clin. Toxicol. 36, 465: A data base search was performed in Washington state for pesticide related illnesses, specifically directed at metal phosphide fumigants such as Aluminium phosphide, but also producing information on zinc phosphide and other pesticide related incidents. The analysis of cases by nature, route of exposure, and clinical outcome yielded the following: Between 1992 and 1996, a total of 2759 cases of possible pesticide-related illnesses were reported, of which 43 % (1186) were related to fumigants. The most common fumigant to which exposure was recorded was, among others, aluminium phosphide with 12 incidents (15 cases). For zinc phosphide, only 3 incidents (3 cases) were reported. Symptoms varied by fumigant and included irritation (mucous membrane and/or skin) and respiratory symptoms for the majority of exposures, but no fatalities occurred. Despite established regulations and training of fumigant applicators, exposures to fumigants continue to occur. Of particular interest are the relatively high number of aluminium phosphide exposures, and the substantial number of exposures due to reentry.

Hayes (1982, TOX96-52228): A 19-year-old woman in her 30th week of pregnancy had ingested (in a drunken state) an unknown amount of zinc phosphide in form of rodenticide bait, subsequently lost consciousness and was cyanotic when brought to the hospital. Following gastric lavage etc., complete recovery was noted after 3 days, and her later born baby appeared normal at birth and subsequent development.

Gosselin et al. (1984, TOX2002-199): A review of the clinical symptoms encountered in cases of poisoning is presented. To date (1984), a total of 25 deaths in humans were known from European literature. Whereas the ingestion of 4-5 g had produced death in human adults (2 cases), even doses as high as 25 – 50 g have been survived by 2 victims. Earliest symptoms involved nausea with vomiting, abdominal pain, tightness in the chest, excitement, agitation and chills. Sometimes rapid exodus occurred. Induced emesis was considered to produce more effective removal of the powder than gastric lavage. Early dyspnoea, shock, oliguria, metabolic acidosis, hypocalcaemic tetany, convulsions, coma were further symptoms. In fatal cases, death occurred after 30 h. Survivors were jaundiced due to extensive liver damage. Most patients were out of danger after 30 h.

Rodenberg et al. (1989, TOX2002-20): Case report: 36 year old male, ingestion of unknown amount of zinc phosphide, suicide intention, ingestion of rodenticide zinc phosphide. Symptoms: nausea, vomiting, dyspnoea, changes in mental status; immediate death from pulmonary oedema.

Sarma and Narula (1996, TOX2006-26): Case report: A 21-year-old woman was hospitalised six hours after ingestion of Zinc phosphide, when she appeared drowsy but her vital signs were normal. Twelve hours after ingestion, she was found to be stuporous, but irritable, febrile (37.4°C), jaundiced and hypotensive. Signs of metabolic acidosis and peripheral circulatory failure were present. Several haematological and clinical chemistry parameters were increased, and generalised ileus with air-fluid levels and an oedematous enlarged pancreas were observed. This acute pancreatitis was successfully managed with conservative treatment. The patient fully recovered within two month after discharge.

Halloran & Reich (1970, TOX2002-202): Case report: 37-year-old man was exposed to zinc phosphide and DDT over a period of 6 months while working 5-days a week in the demolition of a building which had previously been treated with these pesticides. Frequent exposure with pesticide dust working without mask was encountered, finally leading to symptoms such as acneiform eruption, loose prune-juice like stools, nausea, daily vomiting, and after 6 months, loss of eyesight, and bilateral optic atrophy.

The author of this report himself was unable to find any reference linking the exposure to the two compounds to any such effect. In view of the combined exposure, this isolated case report does not allow allocating this phenomenon to any specific effect of zinc phosphide.

B.6.9.3 Clinical signs and symptoms of poisoning

Balali-Mood (1991, TOX2006-185); Childs and Coates (1971, TOX2006-170): Initial clinical manifestations of mild phosphine inhalation mimic an upper respiratory tract infection. Other symptoms may include nausea, vomiting, diarrhoea, headache, fatigue and dizziness. In severe exposure, lung irritation with persistent coughing, ataxia, paraesthesia, tremor, diplopia and jaundice may also occur. Very severe cases may progress to acute pulmonary oedema, cardiac dysrhythmias, convulsions, cyanosis and coma. Oliguria, proteinuria and finally anuria may be induced. Deliberate ingestion of phosphides, especially AID (Phostoxin), causes nausea, vomiting, and sometimes diarrhoea, retrosternal and abdominal pain, tightness in the chest and coughing, headache and dizziness. In severe cases, gastrointestinal haemorrhage, tachycardia, hypotension, shock, cardiac arrhythmias, hypothermia, metabolic acidosis, cyanosis, pulmonary oedema, convulsions, hyperthermia and coma may occur. Clinical features of renal insufficiency and hepatic damage including oliguria, and jaundice may develop later, if the patient does not die. Death, which may be sudden, usually occurs within four days but may be delayed for one to two weeks. Postmortem examinations have revealed focal myocardial infiltration and necrosis, pulmonary oedema and widespread small vessel injury. Chronic poisoning from inhalation or ingestion of phosphine/phosphides may cause toothache, swelling of the jaw, necrosis of mandible, weakness, weight loss, anaemia, and spontaneous fractures. The diagnosis of phosphine poisoning is easy, but the clinical manifestations of phosphine and the phosphides may be similar to those of other toxic chemicals such as arsenic sulphide and calcium oxide. A silver nitrate-impregnated paper test can be used for the breath and gastric fluid of the patients exposed to phosphine/phosphide: silver nitrate and phosphine/phosphides react to form silver phosphide which confirms the diagnosis. Other laboratory investigations such as cell blood counts, haemoglobin, haematocrit, arterial blood gas analyses, renal and liver function tests

and cardiopulmonary monitoring and investigations (ECG and chest X-ray) are essential for the assessment of organ effects and the management of phosphine/phosphide poisoning.

Chugh et al. (1991, TOX2005-305): A total of 418 patients with aluminium phosphide poisoning admitted during January 1981 to December 1987, were studied and analysed for various parameters. Diagnosis of ALP poisoning was based on the history of intake, presence of gastrointestinal symptoms, shock (systolic Bp < 90 mm Hg), and confirmed by a positive bedside silver nitrate impregnated paper test.

The patients showed varied clinical features as shown in Table B.6.9-14.

Table B.6.9-14: Clinical spectrum of aluminium phosphide poisoning

	No. Of patients	%
Gastrointestinal upset, nausea, epigastric burning, retching, pain, etc.	381	91.2
Clear mentation with restlessness, anxiety at admission	381	91.2
Shock	376	90.0
Signs of sympathetic overactivity (sweating, tachycardia)	278	66.5
Oliguria	214	51.2
Tachypnoea, dyspnoea, crepts and rhonchi	192	45.8
Acute renal failure (raised urea and NPN and serum creatinine etc.)	32	7.6
Hepato-biliary (tender hepatomegaly, raised SGOT/SGPT; jaundice)	18	4.3
Bradycardia	14	3.3

All patients were treated similarly with dopamine infusion (starting dose 4 – 8 µg/kg/min), intravenous glucose drip (2 – 3 L glucose saline in first 4 – 6 h), continuous O₂ administration, and systemic corticosteroids. Frequent electrocardiographic monitoring showed varied pattern of ST-T changes, conduction and rhythm disturbances (see Table B.6.9-15).

Table B.6.9-15: Electrocardiographic changes

ECG abnormalities (160 patients)	38.2%
ST-T changes (elevation or depression) in more than 2 leads	56
Varied sino-atrial conduction (sino-atrial block, sinus pauses)	20
Atrial fibrillation or atrial premature beats	14
Bradycardia	14
Bundle branch block: LBBB	6
RBBB	4
Ventricular tachycardia	3
Pericarditis (elevation with ST-T upwards)	3

The mortality was high (77.2 %) and directly related to the dose of poison consumed. The bad prognostic indices and presence of complications further increased the mortality. The mortality did not have any relation with duration and time interval between ingestion and admission. Histopathological changes: lungs, liver and heart showed oedma and congestion, cellular hypoxia. In addition in liver, kidney and heart, areas of necrosis were observed.

Guale et al. (1994, TOX2002-205):

The verification of zinc phosphide poisoning is usually first based on the detection of phosphine from stomach contents or vomitus. For this purpose, Dräger tubes causing a colorimetric reaction in the presence of phosphine are used, combined to 1000 mL squeeze bottle and concentrated hydrochloric acid, bottle with T-tube fitted with stopcocks (air-tight). As a standard, zinc phosphide powder is used. A 25 g sample is weighed and placed in the squeeze bottle (with closed valves), 2 mL of hydrochloric acid are added, followed by immediate closure of the bottle and agitation. The release of phosphine begins immediately,

and after 30 min. The phosphine is distributed in equilibrium between the air space and the aqueous phase. By opening the valve leading to the Dräger tube, and squeezing of bottle until no air is left in the head space (with 10 consecutive repeats), phosphine can then be read from the Dräger tube. The detector tubes have 2 layers, one light-blue pre-layer and a white indicating layer, that changes from white to grey-violet colour upon reaction with phosphine. In addition, by reading the length of discolouration in the tube against a calibrated graduation, phosphine can be quantified with a minimum detection limit of 0.1 ppm. This method was specifically developed for the detection of zinc phosphide poisoning. Different species, with and without addition of different amounts of hydrochloric acid, were tested. It should be noted that in ruminants, the rumen contents with a pH of 5.4 did not release sufficient PH_3 for the detection without addition of extra acid. Hence, the addition of 2 mL of hydrochloric acid in general seemed to be necessary to guarantee adequate liberation of phosphine and detection by the Dräger tubes. The method is highly specific and sensitive, the possibility of false negative results being negligible.

Reigart and Roberts (1999, TOX2002-207):

Summary of data on the recognition and management of Zinc phosphide poisoning. Symptoms: The inhalation of Zinc phosphide dust may induce pulmonary oedema. The emetic effect of zinc released in the gut may provide a measure of protection, but will be alongside the release and absorption of phosphine in the gut together with the zinc. Symptoms include nausea, vomiting, excitement, chills, chest tightness, dyspnoea, cough, and finally lung oedema. Systemic toxicity is comparable to that of yellow phosphorous: hepatic failure, jaundice, haemorrhage, delirium, convulsions, coma (from toxic encephalopathy), tetany from hypocalcaemia, anuria from renal tubular damage, ventricular arrhythmia from cardiomyopathy, and shock are common causes of death. Diagnosis: Confirmation of poisoning is usually by the foul odour of vomitus, faeces, or breath, the luminescence of vomitus or faeces, and hyperphosphataemia and hypocalcaemia occurring in some cases. Treatment: Skin decontamination is by brushing or scraping non-adherent phosphorous (Zinc phosphide) from the skin. Wash skin burns with copious amounts of water. Make sure all particles have been removed. Supportive management of poisonings by Zn_3P_2 is extremely difficult, and needs to be supportive and symptomatic. The control of airways and convulsions must be established prior to considering gastrointestinal decontamination. Caution is warranted since highly toxic phosphine gas may evolve from emesis, lavage fluid, faeces of victims, so ensure good ventilation of patient rooms. Attending persons should wear gloves and avoid contact with the poison. Lavage with potassium permanganate solution (1:5000) is often used, but not recommended by these authors (lack of effectiveness). Catharsis is not indicated, but there may be benefit in administering mineral oil (100 mL for adults; 1.5 mL children < 12 years), but do not give vegetable oils or fats. Transfusions may be required to combat shock and acidosis, i.e. with transfusions of whole blood and appropriate intravenous fluids. Regular monitoring should involve blood electrolytes, glucose, and pH to guide the choice of intravenous solutions. Administration of 100 % oxygen by mask or nasal tube may also be indicated. For renal protection, urine albumin, glucose, and sediment should be monitored to detect early renal injury. Initiate extra corporal haemodialysis in case of acute renal failure, but avoid enhancement of phosphorous excretion. Monitor ECG to detect myocardial impairment. Liver damage is controlled via serum alkaline phosphatase, LDH, ALT, AST, prothrombin time, bilirubin to evaluate liver damage. Administer Vitamin K_1 if prothrombin level declines. Pain management may require morphine sulphate if necessary (adult dose: 2-15 mg/ IM/IV/SC Q 2-6 h; child dose: 0.1-0.2 mg/kg/dose Q 2-4 h).

B.6.9.4 First aid measures

Concerning first aid measures and therapeutic regimes see safety data sheet. The following proposes have been submitted by applicants.

Balali-Mood (1991, TOX2006-185): First aid measures for cases of poisoning with phosphine should comprise standard decontamination measures and symptomatic treatment of non-specific symptoms. No antidote is available for phosphine/phosphide poisoning. Early recognition and management of the poisoning is essential.

Ingestion: In case of ingestion, after consideration of tracheal intubation, perform gastric aspiration and lavage with cold water and preferably sodium bicarbonate solution (2 %). Do not give milk, fats or saline emetics. Administration of repeated doses of activated charcoal through the gastric tube may be useful.

Inhalation: Remove the patient from exposure site, and keep at rest. If the patient is unconscious and breathing stops, immediately ventilate artificially and if the heart stops, begin cardiopulmonary resuscitation.

Monitor and support vital functions, particularly cardiopulmonary, G.I., renal and hepatic functions. Treat shock conventionally and correct acidosis based on blood gas analyses.

Shadnia et al. (2005, TOX2006-192): There is no known antidote for aluminium phosphide intoxication, but in one case it was shown that rapid prevention of absorption by coconut oil might be helpful. In the present case, the same protocol was used in a 28 year old man who had ingested a lethal amount (12 g) of aluminium phosphide with suicidal intent and was admitted to hospital approximately 6 hours post-ingestion. The patient had signs and symptoms of severe toxicity, and his clinical course included metabolic acidosis and liver dysfunction. Treatment consisted of gastric lavage with potassium permanganate solution, oral administration of charcoal and sorbitol suspension, intravenous administration of sodium bicarbonate, magnesium sulphate and calcium gluconate, and oral administration of sodium bicarbonate and coconut oil. Conservative and supportive therapy in the Intensive Care Unit was also provided. The patient survived following rapid treatment and supportive care. It is concluded that coconut oil has a positive clinical significance and can be added to the treatment protocol of acute aluminium phosphide poisoning in humans.

B.6.9.5 Therapeutic regimes

Gastric lavage, with tracheal intubation if appropriate. **Chopra et al. (1988, TOX2007-173); Khosla et al. (1988, TOX2007-170); Misra et al. (1988, TOX2006-182)** applied potassium permanganate for gastric lavage. Coconut oil and activated charcoal may limit absorption of phosphine and may be administered by mouth or stomach tube (although activated charcoal did not work in the patient reported by **Stephenson, (1967, TOX9652073)**). Oral administration of sodium bicarbonate and coconut oil was used successfully in a poisoning case by **Shadnia et al. (2005, TOX2006-192)**. Repeated doses of activated charcoal together with sorbitol (to avoid constipation) may be useful and has been used by the author but has not been yet reported for phosphine/ phosphide poisoning.

Monitor and support vital functions, particularly cardiovascular, respiratory, hepatic and renal functions. Treat shock conventionally (**Chopra et al., (1988, TOX2007-173; Khosla et al., 1988, TOX2007-170)**). Dopamine and hydrocortisone succinate have been used to overcome the shock. Perform arterial blood gas analysis and correct respiratory dysfunction by clearing the airways, giving oxygen and perform artificial (mechanical) respiration if required. Metabolic acidosis must also be treated by giving sodium bicarbonate according to the results

Dermal exposure: Remove any rests by brushing; only then use water for cleansing (in addition to the above mentioned points). Eye contact: Remove rests of preparation with fluff-free cloth; rinse with plenty of water and apply eye drops only after no more powdery residues are visible (in addition to the above mentioned points). Special aids required for First Aid measures: Have methyl prednisolon (application by physician) and a dexamethason spray available. **Suman and Savani (1999, TOX2006-227):** A case report describes resuscitative measures after accidental ingestion of aluminium phosphide tablet. Gastrointestinal upset and shock were treated with two bolus injections of Ringer lactate (20 mL/kg each) along with oxygenation. Gastric wash was done with potassium permanganate solution. Activated charcoal tablets were administered and left in the stomach. Treatment with hydrocortisone and ceftriaxone also started. One hour after treatment symptoms like blood pressure, cyanosis and respiratory distress improved.

B.6.9.6 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion

Singh et al. (1985, TOX2007-177); Chopra et al. (1988, TOX2007-173); Khosla et al. (1988, TOX2007-170): The mortality of attempted suicide by acute phosphine/phosphide poisoning is 37 to 80 % in suicidal patients. However, in occupational or accidental exposure to phosphine, the mortality is much lower and depends on the severity of exposure, age and other predisposing factors of the patients. Death, which may be sudden, usually occurs within four days but may be delayed for one to two weeks. Acute metal phosphide poisoning, particularly deliberate aluminum phosphide poisoning, may cause death within a few hours. Deliberate ingestion of aluminum phosphide, is usually more severe than occupational phosphine intoxication. However, the clinical severity of phosphine/phosphide poisoning could be classified as follows. Mild exposure may present as slight respiratory, gastrointestinal and neuropsychiatric disorders such as cough, shortness of breath, nausea, vomiting, headache, fatigue and dizziness. Moderate exposure may cause cardiovascular, renal and hepatic dysfunction, as well as more severe respiratory, gastrointestinal and neuropsychiatric involvement, e.g. tachycardia, hypotension, persistent coughing, paraesthesia, tremor, diplopia, ataxia, intention tremor, retrosternal and abdominal pain, shortness of breath, oliguria, jaundice and diarrhoea. Severe exposure may progress to shock, gastrointestinal haemorrhage, pulmonary oedema, cardiac arrhythmias, metabolic acidosis, cyanosis, convulsions and coma. Renal failure and liver damage may also occur. Common causes of death following phosphine/phosphide poisoning are pulmonary oedema, cardiac arrhythmias and myocardial injury. A secondary cause of death may be renal failure.

Stephenson (1967, TOX96-52073) classified patients seriously poisoned by phosphine into 3 groups: (a) those who die within a few hours with pulmonary oedema (b) the majority of fatal cases who die after about 30 hours, and (c) those who survive the first 3 days who may not be in danger, despite extensive liver damage and renal dysfunction.

Child and Oates (1971, TOX2006-170) summarised the effects of phosphine on man as follows: see Table B.6.9-16.

Table B.6.9-16: Effects of phosphine on man

Effect	Concentration	
	mg/L	ppm
Rapidly fatal	2.8	2000
Death after 0.5 – 1 hr exposure	0.56 – 0.84	400 – 600
Dangerous to life after 0.5 – 1 hr	0.4 – 0.6	290 – 430
Max. Exposure for 0.5 – 1 hr without serious effects	0.14 – 0.26	100 – 190
Serious effects after several hours	0.01	7
Limit of perceptibility	0.002 – 0.004	1.4 – 2.8

B.6.9.7 Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

Stephenson (1967, TOX96-52073) reviewed the European cases by suggesting that early vomiting improves the prognosis. Two young women swallowed similar quantities of zinc phosphide in a suicide pact. One woman was induced to vomit by mechanical means shortly after poisoning; she had only transient symptoms and recovered completely. Her friend would not vomit and despite gastric lavage one hour after poisoning, she died within 24 hours. Early recognition and treatment of phosphine/phosphide poisoning is therefore of great importance.

Misra et al. (1988, TOX2005-307): Eight cases of phosphine poisoning following ingestion of aluminium phosphide tablets for suicidal attempt are described. The clinical picture consisted of gastritis, altered sensorium and peripheral vascular failure in all cases, cardiac arrhythmia (3), jaundice and renal failure (1 each). Six patients died, the mean hospital stay was 19 h (range 4 – 72).

Post-mortem examination was performed in two patients, revealing pulmonary oedema, gastrointestinal mucosal congestion, petechial haemorrhages on the surface of liver and brain. Histopathological changes included pulmonary oedema, desquamation of the lining epithelium of the bronchioles; vascular degeneration of hepatocytes, dilatation and engorgement of hepatic central veins, sinusoids and areas showing nuclear fragmentation.

Table B.6.9-17 summarises the clinical picture of oral aluminium phosphide poisoning patients.

Table B.6.9-17: Clinical symptoms of oral aluminium phosphide poisoning patients

Patient no.	Age/Sex	No. Of tablets taken	Clinical features	Remarks
1	14/F	1	Gastritis, breathing difficulty, PVF**	Discharged, day 2
2*	31/M	2	Vomiting, coma, PVF**	Died, 22 h
3	19/M	0.5	Gastritis, PVF**	Discharged, day 5
4*	26/M	20	Vomiting, drowsy, PVF**	Died, 5.5 h
5	25/M	4	Vomiting, drowsy	Died, 2 h
6	25/M	2	Vomiting, unconscious	Died, 2 h
7	24/F	?	Vomiting, unconscious	Died, 5.5 h
8	20/F	3	Vomiting, delirium, PVF**, renal failure, jaundice, ventricular tachycardia	Died, 72 h

∅. subjected to autopsy

** peripheral vascular failure

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ArfD and drinking water limit (Annex IIA 5.10)

B.6.10.1 Absorption, distribution, excretion and metabolism

Metal phosphides in contact with moisture (GI tract) readily decompose to metal hydroxide and phosphine, the toxicological principle. Due to the decomposition by moisture other phosphides are regarded as adequate model compounds.

Studies concerning absorption, distribution, metabolism and excretion of ingested zinc phosphide and phosphine are available (Table B.6.10-1). Once formed from the metal phosphide, phosphine is rapidly and completely excreted by exhalation or via urine after oxidation to hypophosphite or phosphite. The phosphine metabolites hypophosphite or phosphite are regarded as less toxic than phosphine itself. Due to the inorganic nature of the metal phosphides and its degradation products and their respective metabolites it is reasonable to assume that residues of these phosphides are expected to be minimal or non-existent.

Following oral administration of zinc phosphide, [³²P] was rapidly absorbed from the gastrointestinal tract. Inhaled PH₃ is considered to be rapidly and quantitatively absorbed through the lungs. [³²P] was detectable in all organs and tissues, with temporary higher levels in liver and medulla oblongata. PH₃ is excreted as such with the expired air or, after metabolic oxidation, with the urine in the form of hypophosphite and phosphite.

In the absence of experimental data, for dermal absorption of both calcium phosphide and PH₃ a default value of 10 %, based on expert judgement, was assumed.

Table B.6.10-1: Summary of toxicokinetic studies

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results	Reference
No guideline; Non-GLP	Oral	Rats, number, bw and sex not stated	Zinc phosphide 40 mg/kg bw (> LD ₅₀) and lower dose (not specified), single application	Mortality↑ at high dose, PH ₃ detectable in liver	Curry, A.S. et al. (1959) (TOX2002-163)
		Rats, sex not stated, 6 animals	Zinc phosphide 10 mg/rat, single application	Mortality↑, phosphide and PH ₃ detectable in liver	
		Rats and guinea pigs, no further information given	No information given	Urinary excretion: main product is hypophosphite	

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results	Reference
No guideline, Non-GLP	Oral, sub-cutaneous, per rectum	Rattus norvegicus Berk, number, bw and sex not stated	Zinc phosphide, [^{32}P]-labelled 40 mg/kg bw	Oral application: After 6-8 h, ^{32}P was detectable in all organs and tissues with temporary higher levels in liver and medulla oblongata. Application per rectum: After 24 h ^{32}P was detectable in large intestine, arterial blood, liver and kidneys. Subcutaneous injection: After 24 h ^{32}P was detectable only around the point of injection.	Andreev, S.B. et al. (1958) (TOX2002- 165)
	Oral		Zinc phosphide, ^{32}P - and ^{65}Zn - labelled Sublethal, lethal, 2-, 3- and 4-fold lethal doses	The distribution of ^{32}P was similar to that in the above experiment. ^{65}Zn was found in all organs. The ratio of ^{32}P to ^{65}Zn was different in different tissues.	
No guideline, Non-GLP	oral	Human	Unknown quantity of Phostoxin tablets	Residues post mortem in stomach, blood, liver	Chan, L.T.F. (1983) (TOX98-50056)
Not applicable	Inhalation			Inhaled PH_3 is considered to be readily absorbed through the lungs, excretion with urine as hypophosphite and phosphite and via lungs as PH_3	WHO (1988) (TOX2005-1201)

B.6.10.2 Acute toxicity

No acute oral toxicity study for calcium phosphide has been submitted by the applicant and no justification was given for that. However, there exist respective studies with other phosphides (summary see Table B.6.10-2). Metal phosphides in contact with moisture (GI tract) readily decompose to metal or calcium hydroxide and phosphine, the toxicological principle. Due to the decomposition by moisture other phosphides are regarded as adequate model compounds. Studies with aluminium phosphide and magnesium phosphide are available and are considered to be of high toxicity when administered orally to animals. Therefore calcium phosphide has to be classified as 'very toxic if swallowed' (T+; R 28). PH_3 , which is developed after contact of calcium phosphide with water by spontaneous hydrolysis of the phosphide, is very toxic by inhalation. According to Annex I to Directive 67/548/EEC classification and labelling of the gas is appropriate (T+; R 26), but calcium phosphide itself is like aluminium phosphide not classified with regard to inhalation toxicity.

No dermal toxicity study for calcium phosphide has been submitted. However, regarding calcium phosphide no higher acute dermal toxicity than observed in aluminium phosphide e.g. is expected (LD_{50} 460 – 900 mg/kg bw). Therefore, for calcium phosphide classification as 'harmful in contact with skin' (Xn; R 21) is required.

No skin irritation study for calcium phosphide has been submitted. However, calcium phosphide reacts like aluminium and zinc phosphide. For both substances no irritation was

noted after application to the skin of rabbits. Therefore, for calcium phosphide no classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is required, too.

No eye irritation study for calcium phosphide has been submitted. However, studies for aluminium and zinc phosphide revealed no eye irritation potential. Therefore, for calcium phosphide no classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is required.

No skin sensitisation study has been presented using calcium phosphide. However, the study for zinc phosphide revealed no skin sensitisation potential. Therefore, calcium phosphide is considered to be not a sensitising substance, too, and classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is not required.

Table B.6.10-2: Overview on the acute toxicity of metal phosphides

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value LD ₅₀ /LC ₅₀	Risk Phrase Remarks	Reference
Acute oral toxicity. Similar to OECD 401 Non-GLP	Rat, Wistar albino 5M+5F	Aluminium phosphide 7.94-8.92-10.0- 11.2 mg/kg bw	LD ₅₀ M+F: 8.7 mg/kg bw	R 28	Sterner, W. and Stiglic, A. (1977) (TOX2006- 981)
Acute oral toxicity OPPTS 870.1100	Rat Wistar, 3M + 3F	Aluminium phosphide 5 mg/kg bw	LD ₅₀ M+F: \approx 5 mg/kg bw	R 28	Stephen, F. (2000) (TOX2006- 210)
Acute oral toxicity. Similar to OECD 401, Non-GLP	Rat Wistar, 5 M + 5 F	Aluminium phosphide 0-6-9-13.5 mg/kg bw	LD ₅₀ : 9 mg/kg bw	R 28	Joshi, M. (1998) (TOX2006- 211)
Acute oral toxicity. OECD 401	Mouse, NMRI/HAN Bö 5M+5F	Aluminium phosphide 6.81-10.0-14.7- 21.5 mg/kg bw	LD ₅₀ M+F: 14.8 mg/kg bw	R 28	Leuschner, J. (1992) (TOX2005- 308)
Acute oral toxicity. Similar to OECD 401, Non-GLP	Mouse CD, 5M + 5F	Aluminium phosphide 0, 9, 11.2, 14 mg/kg bw	LD ₅₀ M+F: 12 mg/kg bw	R 28	Joshi M. (1998) (TOX2006- 212)
Acute oral toxicity No guideline Non-GLP	Rat	Zinc phosphide No dose levels mentioned	LD ₅₀ : 12 mg/kg bw	R 28	Lewis, R.J. (Eds.) (2000): (TOX2006- 19)
Acute oral toxicity No guideline Non-GLP	Rat, CFT-Wistar albino 10F	Zinc phosphide 21, 28, 38, 51, 68 mg/kg bw	LD ₅₀ F: 43 – 56 mg/kg bw	R 25	Krishnakumari , M. et al. (1979) (TOX2002- 167)
Acute oral toxicity No guideline Non-GLP	Deer mice 6 animals	Zinc phosphide	ALD: 42 mg/kg bw	Not acceptable	Schafer, E.W. and Bowles, W.A. (1985) (TOX2002- 168)

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value LD ₅₀ /LC ₅₀	Risk Phrase Remarks	Reference
Acute oral toxicity. No guideline Non-GLP	Rat, Wistar albino 5M+5F	Magnesium phosphide 0.897, 1.0, 1.13, 1.26 g/kg bw total amount	LD ₅₀ M: 20.7 mg/kg bw F: 10.4 mg/kg bw M+F: 11.2 mg/kg bw	R 28	Sterner, W., Chibanguza, G. (1980) (TOX2000- 89)
Acute dermal toxicity. OECD 402	Rat, Wistar albino 5M+5F	Aluminium phosphide 500-1000-2000 mg/kg bw	LD ₅₀ M+F: 900 mg/kg bw	R 21	Dickhaus, S., Heisler, E. (1987) (TOX2000- 93)
Acute dermal toxicity OPPTS 870.1200	Rat Wistar, 5 F/each level + 5 M/highest level	Aluminium phosphide 0-280-420-630 mg/kg bw	LD ₅₀ : 461.2 mg/kg bw	R 21	Stephen, F. (2000) (TOX2006- 213)
Acute dermal toxicity. No guideline Non-GLP	Rat Wistar, 5M+5F	Aluminium phosphide 0-637.7-1275-2550 mg/kg bw	LD ₅₀ : 901 mg/kg bw	R 21	Joshi, M. (1998) (TOX2006- 214)
Acute dermal toxicity Comparable to 92/69/EEC, B3	Rat, Wistar albino 5M+5F	Zinc phosphide 1000-2000-4000 mg/kg bw	LD ₅₀ M+F: 1000 mg/kg bw	R 21	Dickhaus, S., Heisler, E. (1980) (TOX2002- 172)
Acute dermal toxicity Comparable to 92/69/EEC, B3	Rat, Wistar albino 5M+5F	Zinc phosphide 100-200-400-1000 mg/kg bw	LD ₅₀ M+F: 525 mg/kg bw	R 21	Dickhaus, S., Heisler, E. (1980) (TOX2002- 173)
Acute inhalation toxicity. No guideline, non GLP	Rat Wistar, 5M+5F	Aluminium phosphide 0-15.4-26-47 ppm	LC ₅₀ : 34.6 ppm (0.048 mg/L)	R 26	Roy, B.C. (1998) (TOX2006- 215)
Inhalation whole body 6 h exposure, US EPA	Rat Fisher 344	PH ₃ 2.4-4.9-11 ppm	LC ₅₀ : >11 ppm (M/F) equivalent to >0.015 mg/L or >0.675 mg/kg bw	R 26	Newton, P.E. (1989) (TOX97- 51198)
Acute inhalation toxicity, whole body, 1 h exposure. Similar to OECD 403 Non-GLP	Rat, Slc:SD 10M+10F	PH ₃ , developed from magnesium phosphide 150-165-182-200- 220-242 ppm	LC ₅₀ : 204/179 ppm (M/F) equivalent to ⁽²⁾ : 0.29/0.25 mg/L air (M/F) or ⁽³⁾ : 12.9/11.4 mg/kg bw (M/F)	(R 26, PH ₃) ⁽¹⁾	Shimizu, Y. et al. (1982) (TOX2005- 280)
Acute skin irritation, Partly OECD 404	Rabbit, White New Zealand, 5 (sex not mentioned)	Aluminium phosphide 0.5 g/animal	Not irritating	None	Dickhaus, S. and Heisler, E. (1987) (TOX2000- 94)

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value LD ₅₀ /LC ₅₀	Risk Phrase Remarks	Reference
Acute skin irritation. No guideline, non-GLP	Rabbit, White New Zealand, 3M+3F	Aluminium phosphide 0.5 g/animal	Non-irritant	None	Joshi M. (1998) (TOX2006-216)
Acute skin irritation, OECD 404	Rabbit, New Zealand White 3M	Zinc phosphide 0.5 g/animal	Not irritating	None	Brunt, P. (2001) (TOX2005-168)
Acute eye irritation OECD 405	Rabbit White, New Zealand 6 (sex not mentioned)	Aluminium phosphide 0.1 g/animal	Non-irritant (washed out 30 seconds after application)	Study design not suitable	Dickhaus, S. and Heisler, E. (1987) (TOX)2000-95)
Acute eye irritation. No guideline, non-GLP	Rabbit, White New Zealand, 3M + 3F	Aluminium phosphide 1 mg/animal	Not acceptable	Study design not suitable	Joshi, M. (1998) (TOX2006-217)
Acute eye irritation OECD 405	Rabbit, White New Zealand, 2M+1F	Zinc phosphide 0.1 mL/animal	Non-irritant	None	Brunt, P. (2001) (TOX2005-171)
Skin sensitisation OECD 406	Albino Guinea Pig (10M)	Zinc phosphide	Non-sensitising	None	Brunt, P. (2001) (TOX2002-179)

- (1) PH₃ was included into Annex I to Directive 67/548/EEC with R 26, whereas the different phosphides were not classified for inhalation toxicity.
- (2) 1 ppm PH₃ is equivalent to 1.41 µg/L air, density of pure PH₃ (20 °C): (34 g/mol)/(24.1 L/mol) = 1.41 g/L
- (3) Assuming an hourly respiratory volume (rat) of 45 L/(h kg bw)

B.6.10.3 Short term toxicity

Calcium phosphide like other metal phosphides in contact with moisture readily decompose to metal and phosphine, the toxicological principle. Due to the decomposition by moisture other phosphides are regarded as adequate model compounds. Studies with zinc phosphide, aluminium phosphide and phosphine are available. In an oral 90-day gavage test (see Table B.6.10-3), mortality was increased at 2 mg aluminium phosphide/kg bw/d (corresponding to 1.18 mg PH₃/kg bw/d) in both sexes, the NOAEL being 1 mg aluminium phosphide/kg bw/d, equivalent to 0.59 mg/PH₃ bw/d, respectively. However, these values are considered to be of limited reliability due to methodological deficiencies of the respective study report. A subchronic study in a second, non-rodent species was not submitted. An expert statement has been provided: The toxicological profile of calcium phosphide/PH₃ does not differ significantly between rodents and non-rodents and thereby justified non-submission of such data. Male and female rats and mice were exposed up to 0, 1.25, 2.5 or 5 ppm PH₃ for 2 weeks. Under the conditions of this investigation the NOAEL was determined as 2.5 ppm PH₃ (0.95 mg/kg bw/day for rats, 0.1 mg/kg bw/day for mice) based on decreased lung weights in male rats/mice, increased heart weight in female rats/mice and increased urea nitrogen in mice at 5 ppm PH₃ (1.9 mg/kg bw/day for rats, 0.2 mg/kg bw/day for mice). After inhalative administration of up to 3 ppm PH₃ gas (equivalent to ca. 1.1 mg/kg bw/d) to rats over a period of 90 days, no substance related adverse effects were observed. Two satellite groups at 5 and

10 ppm, respectively, were introduced during the course of the study. In the 5 ppm satellite group, which received the test item for only 2 weeks, no relevant effects were observed (which is in accordance with the NOAEL of 4.9 ppm in the inhalative developmental study in rats, see below). Inhalative administration of 10 ppm PH₃ (3.8 mg PH₃/kg/bw/d) was terminated after 3 days, when already 4/10 females had died. In summary, a short-term NOAEL of 1.1 mg PH₃/kg bw/d was established.

Table B.6.10-3: Overview on the short-term toxicity of metal phosphide and phosphine

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value NOAEL	Reference
Subchronic, oral, 13 week, Non-GLP	Rat, CFT- Wistar, 12F (female only)	Zinc phosphide 0, 50, 100, 200, 500 ppm	< 50 ppm (3.5 mg/kg bw/d)	Muktha Bai, K. et al. (1980), (TOX2005-175)
Subchronic, oral, 90 d Non-GLP	Rat, Wistar 24M+24F 32M+32F (control)	Aluminium phosphide 0, 0.1, 0.5, 2 (week 1 and 2) 1 mg/kg bw	1 mg/kg bw (0.59 mg PH ₃ /kg bw)	Schnellhardt, M. et al. (1985), (TOX2005-282)
Subchronic, inhalation, 6h/day, 5d/week, 2 wks, Non-GLP	Rat, Fischer 344; Mouse, B6C3F1, 6M+6F	Phosphine gas (PH ₃) 0, 1.25, 2.5, 5 ppm	2.5 ppm = 0.95 mg/kg bw (rat) 0.0.0.0.0.0.0. (mice)	Morgan, D.L. et al. (1995) (TOX2002-181)
Subchronic, inhalation, 6h/day, 5d/week, 2 – 4 wks, Non-GLP	Mouse, ICR, 10M	Phosphine gas (PH ₃) 5 ppm	No reliable NOAEL can be derived. Study not acceptable	Omae, K. et al. (1996) (TOX2002-174)
Subchronic, inhalation, 6h/day, 5d/week, 13 wks, satellite groups 3 resp. 13 days OECD 413; GLP	Rat, Fischer 344, 30M+30F, satellite 10M+10F and 6M+6F (control)	Phosphine gas (PH ₃) 0, 0.3, 1, 3, satellite groups: 5, 10 ppm	3 ppm = 1.1 mg/kg bw	Newton, P.E. (1990) (TOX2001-684)
Subchronic, inhalation, no guideline, no GLP	Rats (only male), cats and guinea pigs	Phosphine gas (PH ₃) 1, 2.5, 5 ppm No control groups!	No NOAEL can be derived. Study is not acceptable.	Klimmer, O. R. (1969), (TOX96-52057)

1 ppm PH₃ is equivalent to 1.41 µg/L air, density of pure PH₃ (20 °C): (34 g/mol)/(24.1 L/mol) = 1.41 g/L

Assuming an hourly respiratory volume (rat) of 45 L/(h kg bw)

B.6.10.4 Genotoxicity

All submitted *in vitro* bacterial reverse mutation tests (see Table B.6.10-4) showed negative results. No clear result was obtained for the potential of PH₃ to cause clastogenic effects in CHO cells *in vitro*. The results of the test were equivocal, however, the ability of the test design to detect potential clastogenic effects caused by PH₃ could not be demonstrated convincingly. Six submitted *in vivo* tests (see Table B.6.10-5) showed negative results. In a subchronic (13 weeks, mice) *in vivo* test the formation of micronuclei was increased at the highest test concentration (approaching the LD₅₀). However, such exposure conditions are unlikely to be encountered in an occupational environment. In a dominant-lethal-test in mice with aluminium phosphide in peanut oil the post implantation loss was increased and the number of live implants was reduced. In the only dose level also toxic effects have been observed. However, the quality of the study was limited. An inhalative dominant-lethal test in mice was negative. Overall, calcium phosphide/PH₃ is not likely to be genotoxic in humans on relevant exposure conditions.

Table B.6.10-4: Summary of *in vitro* tests

Method	Test system (Organism, strain)	Concentra- tions tested	Results		Reference
			+ S9	- S9	
Bacterial reverse mutation test (Ames test)	Salmonella typhimurium, TA98, TA100, TA1535, TA1537, TA1538, Escherichia coli WP2 Hcr-	0-25600 ppm (estimate)	Negative	Negative	Sutou, S. et al. (1982) (TOX2005-283)
Bacterial reverse mutation test (Ames test)	Salmonella typhimurium, TA98, TA100, TA102, TA1535, TA1537, TA1538	0-4340 ppm	Negative	Negative	Stankowski, L.F. (1990) (TOX2001-685)
Bacterial reverse mutation test (Ames test)	Salmonella typhimurium, TA98, TA100, TA102, TA1537, TA1535	0-1780 ppm	Negative	Negative	Rajwani, L.S. (2000) (TOX2006-220)
Bacterial reverse mutation test (Ames test)	Salmonella typhimurium, TA98, TA100, TA102, TA1537, TA1535, E. coli WP2uvrA	Phosphine gas up to 1 %	Negative	Negative	Araki et al. (1994), (TOX2002-182)
Structural chromosome aberration	CHO-K1-BH4 cells	0-4957 ppm	Equivocal	Equivocal	SanSebastian, J.R. (1990) (TOX2001-686)
Mammalian cell gene mutation (HGPRT test)	V79 hamster cells	0-6580 ppm	Negative	Negative	Leuschner, F. (1992) (TOX2005-284)

Table B.6.10-5: Summary of *in vivo* tests

Method	Species, Strain, Sex, No/sex/group	Route and Frequency of application	Sampling times	Dose levels	Results	Reference
Chromosomal aberration test in mice	Swiss albino mice	Single oral (gavage)	1 day post exposure	0-1.5-3-6 mg/kg bw	Negative	Guna Sherlin, D.M. (1998) (TOX2006-222)
Micronucleus test in mice	Swiss albino mice	2 days, oral (gavage)	1 day after last exposure	0-1.5-3-6 mg/kg bw	Negative	Guna Sherlin, D.M. (1998) (TOX2006-221)
UDS test in rat primary hepatocytes	Rat, CDF (F344)/CrIBR, M, 10	Single whole body inhalation, 6 h exposure time	At 2 and 12-14 h, respectively	0-4.8-13-18-23 ppm	Negative	McKeon, M.E. (1993) (TOX2005-285)
Test for micronuclei	Mouse, Balb-c, M, F, 4-6	Whole body inhalation, 2 weeks, 6 hours/day, 5 days/week	Not indicated	5.5+0.67 ppm	Negative	Barbosa, A. et al (1994) Environ. Mol. Mutagen. 24, 81-88 (TOX97-50676)
	M, F, 12	13 weeks, 6 hours/day, 5 days/week	Not indicated	0-0.3+0.1-1.0+0.2-4.5+0.8 ppm	Positive at the highest concentration	
Test for SCE, chromosome aberrations and micronuclei	Mouse, CD-1 (Charles River), M, 5	6 h inhalative exposure	At 20 hrs. Post-exposure	0-5-10-15 ppm	Negative	Kligerman, A.D. et al. (1994), Environ. Mol. Mutagen. 23, 186 – 189 (TOX97-50677)
Test for SCE, chromosome aberrations and micronuclei	Mouse, CD-1 (Charles River), M, 3-5, Rat, F344/N (Charles River), M, 4-5	6 h/d inhalative exposure on 9 d during an 11 d period.	At 20 hrs. Post-exposure	0-1.25-2.5-5 ppm	Negative	Kligerman, A.D. et al. (1994), Environ. Mol. Mutagen. 24, 301 – 306 (TOX2002-830)
Dominant lethal test	Mouse, B6C3F1 (Charles River), M, 50 (control: 30)	6 h/d inhalative exposure on 10 d during a 12 d period.	-	0-5 ppm		
Test for chromosome aberrations and micronuclei	Mouse (inbred swiss), 4	Zink phosphide, chromosome aberration test: acute: i.p., p.o. and s.c.	24 h post exposure	20-20-40 mg/kg bw	Equivocal, however, study is not acceptable	Pal, B.B., Bhunya, S.P. (1995) (TOX2002-183)
		Subacute: i.p., 5 days		8 mg/kg bw/d		
		Micronucleus test: 2 x i.p.	6 h after last injection	20-30-40 mg/kg bw		
		Sperm abnormality test: i.p., 5 days	35 days after first injection	20-30-40 mg/kg bw		

Method	Species, Strain, Sex, No/sex/group	Route and Frequency of application	Sampling times	Dose levels	Results	Reference
Dominant lethal test	Mouse, Swiss albino, control: 10 M, treated group: 11 M	Aluminium phosphide in peanut oil	-	0-6 mg/kg bw/day	Positive at toxic concentration	Rajesh Sundar, S. (1999) (TOX2006-224)

B.6.10.5 Long term toxicity and carcinogenicity

Phosphine was assessed for chronic inhalation toxicity and carcinogenicity in a combined 104 week study in rats (summary in Table B.6.10-6). The gas was produced by aluminium phosphide. In the inhalation study, body weight, food consumption, routine haematology, serum biochemical, and urinary analyses were all similar to control animals. Ophthalmological observations, gross pathology, organ weights and histopathology indicated no adverse effects from phosphine exposures. The NOAEL was 3.0 ppm, the highest concentration tested. This dose level is equivalent to 1.1 mg/kg bw/day. In two older limited dietary studies, rats received diets treated with phosphine released from aluminium phosphide. Behaviour, general appearance, survival, body weight, food consumption, haematology, blood chemistry, urine analyses and bone smear data, as well as gross and microscopic findings and rate of tumour development, did not reveal any toxic effects from the aluminium phosphide treated diet. However, the test design of both studies was insufficient. Therefore, the oral studies are considered to be not acceptable.

Based on lack of exposure and the absence of genotoxic concern waiving of a long term/carcinogenicity study in a second species was seen as justified.

Table B.6.10-6: Summary of long-term toxicity and carcinogenicity

Study and dose levels (mg/kg/day)	NO(A)EL	LOEL	Reference
Combined rat chronic (2 year) toxicity and carcinogenicity study, 0, 0.3, 1, and 3 ppm by inhalation with purified PH ₃	Toxicity: NOAEL: 3 ppm equivalent to 0.0042 mg/L or 1.1 mg/kg bw/day	Toxicity: LOEL: >3 ppm Based on lack of systemic toxicity at any dose level	Newton, 1998 (TOX2000-98)
	Carcinogenicity: NOEL: 3 ppm	Carcinogenicity: LOEL: >3 ppm based on lack of carcinogenicity at any dose level	
Rat chronic (2 year) toxicity, oral, levels of phosphine in diet after fumigation ranged from 0.167-7.5 mg/kg	No effects observed. However, the study is considered to be not acceptable.	-	Hackenberg, 1969/1972 (TOX96-52058) / (TOX2005-286)
Rat chronic (2 year) toxicity, oral, level of phosphine in diet after fumigation 5 ppb	No effects observed. However, the study is considered to be not acceptable.	-	Cabrol et al., 1985 (TOX2002-831)

B.6.10.6 Reproductive Toxicity

An acceptable two generation reproduction study was not submitted. However, long-term exposure is negligible and there is a very steep dose response curve of metal phosphide / phosphine toxicity from which it can be expected that maternal toxicity would dominate over reproductive effects. Therefore, a waiving concept was accepted. A 2-generation oral study in rats with fumigated diet (fumigation with phosphine) was published. No effects have been observed, however, the study was considered to be not acceptable. The effects of phosphine gas on pregnancy/embryo-foetal development were evaluated in a developmental toxicity study in the rat (summary see Table B.6.10-7). Treatment was by inhalation with phosphine, the gas produced by aluminium phosphide. Phosphine administered by whole body inhalation to pregnant females rats at target exposure levels up to 5.0 ppm for 6 hrs/day over the day 6-15 of gestation interval was not maternally toxic, embryotoxic, foetotoxic or teratogenic. However at the 7.5 ppm exposure, the first 14 mated females on test died during the day 8-15 gestation interval after receiving three to 10 days of exposure. Therefore, the No Observed Effect Level (NOEL) for the maternal and developmental toxicity for this study in rat was 5 ppm. The analytical concentration of this target dose was 4.9 ppm, equivalent to 0.007 mg/L air or 1.9 mg/kg bw/day.

Table B.6.10-7: Summary of maternal and developmental toxicity

Study and dose levels (mg/kg/day)	NO(A)EL	LOEL	Reference
Rat 2-generation study with fumigated diet	No effects in result of fumigation. Concentration of as in diet not measured. The study is not acceptable	No effects in result of fumigation. Concentration of as in diet not measured.	Cabrol, 1989 (TOX2005-189)
Rat developmental toxicity 0, 0.03, 0.3, 3.0, 5.0 and 7.5 ppm (by inhalation)	Maternal toxicity: NOEL: 5 ppm	Maternal toxicity: LOEL: 7.5 ppm Based on mortality	Schroeder, 1989 (TOX2001-687)
	Developmental toxicity: NOEL: 5 ppm * Equivalent to 0.007 mg/L air or 1.9 mg/kg bw/day	Developmental toxicity: LOEL: >5 ppm Up to 5 ppm no developmental tox. Was observed, dose group 7.5 ppm was early terminated	

Ø. = The analytical concentration was 4.9 ppm.

B.6.10.7 Neurotoxicity

The neurotoxicity of phosphine has been assessed in rats in an acute and a 90-day inhalation study (see Table B.6.10-8). In the acute neurotoxicity study, rats were exposed to 0, 20, 30 and 40 ppm phosphine gas (nominal conc.) administered via whole body inhalation exposure for one session of four hours duration. The No Observable Adverse Effect Level (NOAEL) of phosphine in rats was 40 ppm (analytical conc. 38 ppm) with regard to anatomic pathology and the behavioural and neurological status observed in the functional observational battery, and less than 20 ppm with regard to changes in motor activity on day 1. In the subchronic neurotoxicity study, rats were exposed to phosphine gas via whole body exposure at levels of 0.3, 1 and 3 ppm, 6 hours per day, 5 days per week, for 13 weeks. Due to equivocal effects seen in high dose males, and the lack of effects seen in females the No Observed Adverse

Effect Level (NOAEL) of phosphine for systemic/neurotoxic effects in rats exposed over a 90-day period is 3 ppm, the highest dose tested in this study.

Table B.6.10-8: Overview of neurotoxicity studies on phosphine

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels	Value NOAEL	Reference
Rat acute neurotoxicity Whole body inhalation, 4 h US EPA Series 81, 82, 83, 82-4	Rats, Sprague Dawley albino 11M+11F	Phosphine 0, 20, 30, 40 ppm (nominal conc.) (analytical conc.: 0, 21.2, 27.6, and 38.4 ppm)	Motor activity: NOAEL < 21 ppm FOB and histopathology NOAEL = 38 ppm	Schaefer, 1998 (TOX2000-97)
Rat repeat-dose neurotoxicity whole body inhalation, 6h/day, 5d/week, 13 weeks EU 2004/73/EC, B.43 (2004)	Rats, Charles River Crl:CD BR VAF/Plus 16M+16F, recovery 6M+6F	Phosphine 0, 0.3, 1, 3 ppm	Neurotoxic/ systemic NOAEL 3 ppm	Schaefer, 1998 (TOX2006-176)

B.6.10.8 Further toxicological studies

It was demonstrated that phosphine or other phosphide derived reaction products induced Heinz body formation in relatively low concentrations (1.25 ppm) in normal human erythrocytes. The time course for the induction of Heinz bodies is relatively slow (4 h). The formation of Heinz bodies by phosphine is oxygen-dependent, consistent with earlier work regarding the insecticidal properties of the chemical. Finally, these *in vitro* data lead to the speculation that prolonged *in vivo* exposure to phosphine in concentrations exceeding the PEL might have an adverse effect on haemoglobin in susceptible segments of the worker population exposed to the chemical. The results of another study show that after acute poisoning of rats by phosphine the respiration of the isolated liver mitochondria is diminished. The oxidation of α -ketoglutarat turned out to be the most sensitive. The oxidative phosphorylation, however, remains on a normal level. In general, the disturbance equals that of phosphine action on isolated mitochondria *in vitro*. Similar effects have been observed on the isolated sarcosomes of heart muscle of poisoned animals on an early state of intoxication. But in the sarcosome respiration and phosphorylation is uncoupled at the same time. Since the respiration of *Neurospora crassa* is also decreased by phosphine it is to assume that this agent acts by this mechanism on living cells in general. The same kind of disturbance can be demonstrated in the mitochondria after chronic administration of doses which are far below the toxic ones of phosphine and by which animals do not show any sign of damage. There is a small but considerable fall of CoA in the liver of acute poisoned animals.

B.6.10.9 Medical data and information

Among the examined persons, occupied in the production of Polytanol (Calcium phosphide), no health impairment was detected over a period of 3 to 16 years. The case reports submitted by the applicants are considered to be representative of the numerous records of poisoning cases, mainly in connection with suicide, which are available from the literature. Diagnosis is mainly based on the history of intake, gastrointestinal symptoms, shock symptoms and silver nitrate impregnated paper test. Main symptoms are severe circulatory, cardiac, and renal failure, uraemia, hepatic damage, changes in ECG, and respiratory distress connected with a high mortality rate. Histopathological changes have mainly been observed in lungs, liver, heart and kidney. Since an antidote is not available, therapy relies on treatment of the clinical symptoms and administration of high doses of corticoids.

B.6.10.10 Acceptable Daily Intake (ADI)

The most relevant study to derive the ADI is the long term study in rats. The NOAEL in the 2-yr inhalation study was 3 ppm (equivalent to 1.1 mg PH_3 /kg bw/day). In the absence of experimental data, it is assumed, that after ingestion of calcium phosphide, phosphine gas would be liberated stoichiometrically (0.37 g PH_3 /g calcium phosphide) and successively be quantitatively absorbed. Based on the NOAEL for PH_3 of 1.1 mg/kg bw/day, the maximum liberation of gas of 0.37 g PH_3 /g calcium phosphide and a safety factor of 100 an **ADI of 0.030 mg/kg bw/day** is derived.

B.6.10.11 Acceptable Operator Exposure Level (AOEL)

The most relevant study to derive the AOEL is the 90-day inhalation study in rats. The NOAEL in the 90-day inhalation study was 3 ppm (equivalent to 1.1 mg PH_3 /kg bw/day). In the absence of experimental data, it is assumed, that after ingestion of calcium phosphide, phosphine gas would be liberated stoichiometrically (0.37 g PH_3 /g calcium phosphide) and successively be quantitatively absorbed. Based on the NOAEL for PH_3 of 1.1 mg/kg bw/day, the maximum liberation of gas of 0.37 g PH_3 /g calcium phosphide and a safety factor of 100 an **AOEL of 0.030 mg/kg bw/day** is derived.

B.6.10.12 Acute Reference Dose (ArfD)

The most relevant study to derive the ArfD is the developmental study in rats. The NOAEL in the developmental study in rats was 4.9 ppm (equivalent to 1.9 mg PH_3 /kg bw/day). In the absence of experimental data, it is assumed, that after ingestion of calcium phosphide, phosphine gas would be liberated stoichiometrically (0.37 g PH_3 /g calcium phosphide) and successively be quantitatively absorbed. Based on the NOAEL for PH_3 of 1.9 mg/kg bw/day, the maximum liberation of gas of 0.37 g PH_3 /g calcium phosphide and a safety factor of 100 an **ArfD of 0.051 mg/kg bw** is derived.

B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1)

Polytanol is a gas generating product (GE) produced as pellets with a content of 180 g/kg of the pure active ingredient calcium phosphide (Ca_3P_2). The specification of the preparation is given in Annex C. The content of the active substance is calcium phosphide, techn. = 1000 g/kg and calcium phosphide, pure = 180 g/kg, thus as stated by the notifier only impurities and no formulants are to be considered. The maximum purity of the active substance as manufactured is 280 g/kg. (see Table B.6.11-1)

Table B.6.11-1: Preparation with the active ingredient calcium phosphide:

Notifier	Preparation		Content of as
Chemische Fabrik Wülfel	Polytanol	Gas generating product GE as pellets	CaP, techn. 1000.0 g/kg CaP, pure 180.0 g/kg

Only the acute oral and the acute inhalation study has been conducted with Polytanol. For further assessment of the Ca_3P_2 containing product the notifier refers to the zinc phosphide (Zn_3P_2) studies submitted to assess the active substance (Ca_3P_2). The results of the acute toxicity tests with Polytanol or the respective studies submitted/cited by the notifier are summarised in Table B.6.11-2.

Table B.6.11-2: Summary of acute toxicity studies to assess Polytanol

Study [test substance]	Species	Result	Reference
Acute oral toxicity [Polytanol, 17.6 % oily dilution]	Rat	$\text{LD}_{50} = 72.32 \text{ mg/kg bw (M+F)}$	Venugopala Rao, K. (1999) (TOX2000-105)
Acute percutaneous toxicity: [zinc phosphide]	Rat	$\text{LD}_{50} = 1000 \text{ mg/kg bw (M+F)}$	Dickhaus, S., Heisler, E (1980) (TOX2002-172 + TOX2002-173)
Acute percutaneous toxicity:	Rat	(e.g. aluminium phosphide: $\text{LD}_{50} = 460 - 900 \text{ mg/kg bw}$)	(Div. studies – see B.6.2)
Acute inhalation toxicity (4 h) [phosphine generated from Polytanol]	Rat	$\text{LC}_{50} = 64 \text{ ppm (M+F)}^*$ equivalent to $0.090 \text{ mg/L air (M+F)}$ or 16 mg/kg bw^{**}	Venugopala Rao, K. (2001) (TOX2002-822)
Skin irritation [zinc phosphide]	Rabbit	Non-irritating (0.5 g/animal ; 4 h)	Brunt, P. (2001) (TOX2005-168)
Eye irritation [zinc phosphide]	Rabbit	Non-irritating (0.1 mL/animal)	Brunt, P. (2001) (TOX2005-171)
Skin sensitisation [zinc phosphide]	Guinea pig	Non-sensitising	Brunt, P. (2001) (2002-179)

Ø. 1 ppm PH_3 equivalent to 0.00141 mg/L air [PH_3 (20 °C): 34 g/mol ; 24.1 L/mol]

** respiratory volume: 45 L/kg bw/h (rat); 4 h exposure

In contrast to the notifier in this draft assessment report the data for aluminium phosphide (AlP) and zinc phosphide (Zn_3P_2) are used to assess calcium phosphide and Polytanol if no specific studies were available. Since both metal phosphides react with moisture to evolve phosphine gas which is the substance responsible for the toxicity of the product, tests with aluminium phosphide can also be used to assess the toxicity of Polytanol. Based on the acute oral LD_{50} value of Polytanol in rats (72.32 mg/kg bw) classification/labelling with R 25 (toxic

if swallowed) is considered suitable. The relative low value is caused by the concentration of the pure active ingredient in Polytanol (Ca_3P_2 , pure 180 g/kg) in relation to other known metal phosphide containing products with contents of more than 560 g as/kg. The dermal LD_{50} value derived from the study conducted with zinc phosphide and considering further data e.g. from ALP studies leads to classification/labelling with R 21 (harmful in contact with skin). The acute inhalation toxicity of Polytanol should not be classified regarding the LC_{50} value but assessed as phosphides and other phosphide containing products with R 15/29 (contact with water liberates toxic, extremely flammable gas) and R 32 (contact with acids liberates very toxic gas). The potential of Polytanol to irritate skin and eyes and the skin sensitising properties should be assessed on the basis of respective studies conducted with the active ingredients aluminium and zinc phosphide. Calcium phosphide reacts like aluminium and zinc phosphide. Therefore, for the active ingredient calcium phosphide no respective classification/labelling is proposed. For skin irritation and skin sensitisation considering information on further studies also conducted with phosphide containing products (e.g. aluminium phosphide) these results are acceptable for Polytanol, too. Because some information regarding eye irritating properties of phosphide containing products are available and considering the difficulty to investigate such effect if the high toxic phosphine is generated in moisten surroundings, the RMS proposed classification/labelling of Polytanol with R 36 (irritating to eyes). Although some studies are only considered supplementary or insufficient to make a decision regarding the respective classification/labelling, the overall information is considered sufficient to assess the product and for the sake of animal welfare and protection no further studies should be required.

With regard to Directive 1999/45/EC and also considering the similarity of other formulations in discussion, the RMS proposes for Polytanol:

T	Toxic
R 15/29	Contact with water liberates toxic, extremely flammable gas
R 21	Harmful in contact with skin
R 25	Toxic if swallowed
R 32	Contact with acids liberates very toxic gas
R 36	Irritating to eyes

B.6.11.1 Acute oral toxicity – Polytanol

Report:	Venugopala Rao, K. (1999): Acute oral toxicity with Polytanol in Wistar Rats; Toxicology Department Rallis Research Centre, Rallis India Ltd., Bangalore, India; report no.: 2739/99, October 15, 1999. (TOX2000-105)
Guidelines:	OECD 401.
Deviations:	None.
GLP:	Yes (certified laboratory).
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test material: Polytanol (grey pellets: Tricalciumdiphosphide, CAS-No. 1305-99-3) manufactured by Chemische Fabrik Wülfel, Hannover, Germany; batch-No.: 127; purity: 17.6 % w/w; vehicle/solvent: Corn oil. Test animals: Wistar rats; source: Toxicology Department Rallis Research Centre, Bangalore, India; age at start of treatment: 8-10 weeks; body weight at start of treatment: males 234 – 291 g, females 163 – 190 g. Animal allocation

and treatment: 10 Wistar rats (5 males and 5 females) per group were acclimatised seven days under laboratory conditions after veterinary examination.

Table B.6.11-3: Groups, dosing of Polytanol and test item preparation:

Group	Dose (Polytanol) (mg/kg bw)	Test item (mg)	Corn oil (mL)	Concentration (mg/mL)	Dose volume (mL/kg bw)
G1	0	-	x	-	-
G2	60	360	30	12	5
G3	80	480	30	16	5
G4	100	600	30	20	5

The prepared suspensions were administered once orally by gavage to the fasted rats at the dose volume of 5 mL/kg body weight to deliver the doses of 0 (G1), 60 (G2), 80 (G3) and 100 (G4) mg/kg bw. Dead rats were necropsied and the rats surviving to the end of the observation period were sacrificed by ether anaesthesia and necropsied.

Findings:

The results are represented in Table B.6.11-4. The pre-terminal deaths were 0, 20, 70 and 90 % in G1, G2, G3 and G4.

Table B.6.11-4: Results of acute oral toxicity study with Polytanol in Wistar Rats

Group No.	Dose (mg/kg bw)	Toxic signs	No.: dead/tested	Day of death	Mortality (%)
G1	0	-	0/10	-	0
G2	60	Males: - Females: Lethargy and/or recumbency on day 1	2/10	2	20
G3	80	Lethargy and/or ataxia with recumbency in two rats on day 1 with deaths of one male rat and four female rats. On day 2, two male rats died. The surviving rats were lethargic. All the surviving rats were normal from day 3 onwards.	7/10	1 – 2	70
G4	100	Lethargy and/or ataxia with recumbency on day 1 with deaths of three male and four female rats. On day 2, one male and one female rats died. The surviving rat had lethargy. The surviving rat was normal from day 3 onwards.	9/10	1 – 2	90

Lethargy and/or ataxia with recumbency on day 1 were seen in all dosed groups. All the surviving rats were normal from day 3 onwards. All the surviving rats gained weight during the experimental period and all the dead rats had lost weight in all the groups. Lung congestion was observed in some animals in all dosed groups.

Conclusion:

The calculated acute oral LD₅₀ value of Polytanol in Wistar rats is 72.32 mg/kg bw with 95 % confidence limits of 59.33 and 82.47 mg/kg bw. On this basis, classification/labelling with R25 (toxic if swallowed) would be needed.

B.6.11.2 Acute percutaneous toxicity – Polytanol

Justification for non-submission as given by the notifier:

As result it is not considered valid to measure the acute dermal toxicity of Polytanol (calcium phosphide), since contact with dermal moisture will result in rapid decomposition and evolution of phosphine and calcium dihydroxide which will invalidate any attempt at estimation. Dermal absorption of calcium phosphide and phosphine is regarded to be insignificant [see: WHO Environmental Health Criteria No. 73, Phosphine and Selected Metal Phosphides, page 49 (1988)] (TOX2005-1201. The acute dermal LD₅₀ for zinc phosphide in rats has been reported :

LD₅₀ 24 hours 2000 mg/kg bw

LD₅₀ 14 days 1000 mg/kg bw.

For more details please refer to references:

Document K, Section 3, Tier II, Annex IIA, point 5.2.2/02, [Dickhaus, S. (1980)] : and Document K, Section 3, Tier II, Annex IIA, point 5.2.2/03, [Dickhaus, S. (1980)] respectively.

Report : Dickhaus, S. and Heisler (1980): Akute Toxizitätsprüfung von der Substanz “Zinkphosphid” nach dermalen Applikation an der Ratte, report no.: 1-4-258-80. (TOX2002-172)

Guidelines: In consideration of ‘Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics’, Division of Pharmacology, FDA 1959.

Deviations : Batch number, purity not mentioned. No identification if the active substance or a product has been tested. Different information regarding the observation period for mortality were given.

GLP : No.

Acceptability : The study is considered to be supplementary.

The dermal (intact skin) LD₅₀ of zinc phosphide in rats was calculated to be for 24 hours 2000 mg/kg bw and for 14 days 1000 mg/kg bw for both sexes.

Report : Dickhaus, S. (1980): Akute Toxizitätsprüfung von der Substanz “Zinkphosphid” nach dermalen Applikation an der skarifizierten Haut der Ratte, report no.: 1-4-258a-80. (TOX2002-173)

Guidelines : No.

Deviations : Batch number, purity not mentioned. No indication if the active substance or a product has been tested. Different information regarding the observation period for mortality were given.

GLP : No.

Acceptability : The study is considered to be supplementary.

The dermal (scarified skin) LD₅₀ of zinc phosphide in rats was calculated to be 775 mg/kg bw (24 hours) or 525 mg/kg bw (14 days) for both sexes.

Conclusion:

The acute dermal toxicity of metal phosphides differs. Aluminium phosphide is more toxic than zinc phosphide e.g. : The LD₅₀ values were calculated to be 460 – 900 mg/kg bw and therefore classification as ‘harmful in contact with skin’ (Xn; R 21) is required for both substances. Regarding calcium phosphide no higher acute dermal toxicity than observed in

aluminium phosphide is expected. Consequently, calcium phosphide should be classified as “harmful in contact with skin” (Xn; R 21) (see B.6.2).

B.6.11.3 Acute inhalation toxicity – Polytanol

Report :	Venugopala Rao, K. (2001): Acute inhalation toxicity study with Polytanol in Wistar Rats; Toxicology Department Rallis Research Centre, Rallis India Ltd, Bangalore, India; report no.: 3078/00, July 21, 2001. (TOX2002-822)
Guidelines :	OECD 403.
Deviations :	None.
GLP :	Yes (certified laboratory).
Acceptability :	The study is considered to be acceptable.

Material and methods:

Test material: Polytanol (grey pellets: Tricalciumdiphosphide, CAS-No. 1305-99-3) manufactured by Chemische Fabrik Wülfel, Hannover, Germany; batch-No.: 127; purity: 17.6 % w/w; vehicle/solvent: Deionised water. Test animals: Wistar rats; source: Toxicology Department Rallis Research Centre, Bangalore, India; age at start of treatment: 17-18 weeks; body weight at start of treatment: males 355-396 g, females 214-243 g.

Animal allocation and treatment:

10 young adult Wistar rats (5 males and 5 females per group) were acclimatised 7-9 days under laboratory conditions after veterinary examination.

Detail of Exposure:

1. Exposure system: Whole body, static atmosphere condition

2. Phosphine gas analysis:

a) Analysis at breathing zone, using real time phosphine monitor “UNIPHOS 250” (0- 200 ppm range, diffusion type), having LCD display and the real time concentrations recorded at intervals of 5 minutes throughout the exposure period of 4 hours.

b) A personal safety monitor was used to check presence of traces outside the airtight chamber (if any) – “UNIPHOS 350” (0-20 ppm range). There was no reading indicating the absence of any leakage from the chamber.

3. Facilitating phosphine gas liberation

A piece of silicon rubber tubing was connected to one end of a steel tube (facing outer side of chamber) which was inserted into a rubber cork and another piece of silicon tubing was connected to the other end of the steel tube (facing inside the chamber) to drip in the water into the crucible kept inside the chamber in which accurately weighed quantities of Polytanol pellets were placed. The rubber cork (having steel tube and silicon tube connections) was inserted into a hole at the top of the inhalation chamber and fixed firmly to make the chamber airtight.

4. Duration of exposure

Rats were exposed continuously for four hours to the test item gas. The post exposure observation was made once daily for 15 days.

5. Animal restraint during exposure

The rats were individually housed in compartmented stainless steel mesh cages with ten compartments per cage and kept inside the exposure chamber.

Test item preparation:

A quantity of 2.0, 3.0 and 4.0 g of Polytanol was moistened with deionised water to facilitate the liberation of phosphine gas for the groups G1, G2 and G3 groups, respectively.

Treatment:

All the rats of one group were exposed continuously for four hours to gas (phosphine) in the inhalation chamber. Exposure was carried out on three successive days in the same inhalation chamber, i. e. day 1-G1 group, day 2 –G2 group and on day 3 –G3 group.

Dead rats were necropsied and the rats surviving to the end of the observation period were sacrificed by ether anaesthesia and necropsied.

Findings:

The results acute inhalation (4 h) toxicity study with Polytanol in Wistar rats are presented in Table B.6.11-5.

Table B.6.11-5: Results of acute inhalation toxicity study with Polytanol in Wistar Rats

Group No.	Dose		Toxic signs	No.: dead/tested	Day of death	Mortality (%)
	Polytanol (g)*	Phosphine (mg/L air)				
G1	2.00	0.079 (= 57.88 ppm)	Nasal irritation in all the rats; surviving rats were normal from day 2 onwards.	1/10	1 (4 h)	10
G2	3.00	0.096 (=70.44 ppm)	Nasal irritation in all the rats; some animals lethargic; surviving rats were normal from day 2 onwards.	7/10	1 (3 h)	70
G3	4.00	0.099 (=72.50 ppm)	Nasal irritation in all the rats; some animals lethargic; surviving rat was normal from day 2 onwards.	9/10	1 (3-4 h)	90

∅. Polytanol was moistened with deionised water to facilitate the quick release of the phosphine gas.

∅. Test item gas concentration in chamber air

The mean phosphine concentration in the chamber air was 57.88 (0.079 mg/L), 70.44 (0.096 mg/L) and 72.50 (0.099 mg/L) ppm for G1 (low dose), G2 (mid dose) and G3 (high dose) groups respectively as measured by the real time phosphine monitor “UNIPHOS 250”.

2. Mortality (Table B.6.11-5):

Low dose group: One female rat died at 4th hour of exposure on day 1.

Mid dose group: Three male and four female rats died at 3rd hour of exposure on day 1.

High dose group: Four male and all female rats died at 3rd / 4th hour of exposure on day 1.

All the surviving rats gained weight during the observation period except for a female rat of low dose group which had same body weight at interim weighing as compared to its initial weight, however, it gained weight at terminal weighing.

4. Toxic signs:

G1: Nasal irritation was observed in all the rats throughout the exposure period. All surviving rats were normal from day 2 onwards.

G2: Nasal irritation was observed in all the rats during first two hours of exposure period. Two female rats were lethargic at 2nd hour of exposure and two male rats and one female rat were lethargic during 3rd and 4th hour of exposure period. All surviving rats were normal from day 2 onwards.

G3: Nasal irritation was observed in all the rats during first two hours of exposure period. One male rat was lethargic at 3rd and 4th hour of exposure period and two female rats were lethargic during 3rd hour of exposure period. The surviving male rat was normal from day 2 onwards.

5. Necropsy:

G1: There were no abnormalities detected at necropsy.

G2: Lung congestion was observed in one male and one female rat and lung and liver congestion was observed in two male and one female rat at necropsy. There were no abnormalities at necropsy in the remaining rats.

G3: Lung congestion was observed in one male and two female rats and liver congestion was observed in one male and three female rats at necropsy. There were no abnormalities detected at necropsy in the remaining rat.

Conclusion:

The acute inhalation (4 h) LC₅₀ value of phosphine generated by Polytanol in Wistar rats was 0.090 mg/L air with 95 % confidence limits of 0.083 and 0.095 mg/L air. On this basis, classification/labelling with R 26 (very toxic by inhalation) would be needed.

B.6.11.4 Skin irritation – Polytanol

Justification for non-submission as given by the notifier:

Polytanol (calcium phosphide) is a dry granular solid which decomposes very rapidly in contact with water to produce highly toxic gas phosphine. As result it is not considered valid to measure the skin irritation of calcium phosphide, since contact with dermal moisture will result in rapid decomposition and evolution of phosphine and calcium dihydroxide which will invalidate any attempt at estimation. In general skin irritation of calcium phosphide and phosphine is regarded to be insignificant. But this is unlikely with zinc phosphide. The result of tests on skin irritation is, that zinc phosphide is classified as non-irritant according to the classification scheme of Draize. No classification is required according to the classification criteria of EU Commission Directive 93/21/EEC. For more details please refer to reference: Document K, Section 3, Tier II, Annex IIA, point 5.2.4/02 [Brunt, P.(2001)].

Report : (see B.6.2.4)	Brunt, P. (2001): Zinc phosphide: Acute dermal irritation in the rabbit; Safepharm Laboratories, Derby, U.K., project No.: 1483/001, unpublished report, March 1, 2001. (TOX2005-168)
Guidelines :	OECD Guideline 404 'Acute Dermal Irritation/Corrosion' (adopted 17 July 1992); Commission Directive 92/69/EEC Method B4 Acute Toxicity (Skin Irritation).
Deviations :	None.
GLP :	Yes.
Acceptability :	The study is considered to be acceptable.

The test material was classified as non-irritant.

Conclusion:

In contrast to the notifier in this draft assessment report the data available for aluminium phosphide and zinc phosphide are used to assess the active substance calcium phosphide:

"... calcium phosphide reacts like aluminium and zinc phosphide. For both substances no irritation was noted after application to the skin of rabbits. Therefore, for calcium phosphide no classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is required, too" (see B.6.2).

B.6.11.5 Eye irritation

Justification for non-submission as given by the notifier:

Hydrolysis of calcium phosphide (Polytanol) on the eyes would lead to the evolution of gaseous phosphine and the calcium cations. Phosphine could only be absorbed by inhalation. In general eye irritation of calcium phosphide and phosphine is insignificant. But this is unlikely with zinc phosphide. The result of a study on eye irritation is, that zinc phosphide is classified as mild-irritant according to the classification scheme of Kay and Callandra. However, no classification is required according to the classification criteria of the EU Commission Directive 93/21 EEC. For the reason given above it is not considered feasible to conduct irritating studies on eyes with calcium phosphide according to 92/69 EEC. We suggest to preferably refer to the results with zinc phosphide:

Document K, Section 3, Tier II, Annex IIA, point 5.2.5/02 [Brunt, P. (2001)].

Report : (see B.6.2.5)	Brunt, P. (2001): Zinc phosphide: Acute eye irritation in the rabbit; SafePharm Laboratories, Derby, U.K., Project No.: 1483/002, unpublished report, March 1, 2001. (TOX2005-171)
Guidelines :	OECD Guideline 405 'Acute Eye Irritation/Corrosion' (adopted 24 Feb 1987); Commission Directive 92/69/EEC Method B5.
Deviations :	None.
GLP :	Yes.
Acceptability :	This study is considered to be not suitable to assess the eye irritating potential of calcium phosphide.

The test material was classified as mild irritant according to the scheme of Kay and Callandra. However, no classification is required according to the classification criteria of the EU Commission Directive 93/21 EEC.

Conclusion:

In contrast to the notifier in this draft assessment report the data available for aluminium phosphide and zinc phosphide are used to assess the active substance calcium phosphide:

"... studies for aluminium and zinc phosphide revealed no eye irritation potential. Therefore, for calcium phosphide no classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is required." (B.6.2)

Nevertheless, because some information regarding eye irritating properties of phosphide containing products are available (see also draft assessment report to aluminium phosphide) and considering the difficulty to investigate such effect if the high toxic phosphine is generated in moist surroundings, the RMS proposed classification/labelling of Polytanol with R 36 (irritating to eyes).

B.6.11.6 Skin sensitisation – Polytanol

Justification for non-submission as given by the notifier:

Polytanol (calcium phosphide) is a dry granular solid. The particle size is ranging from 2 – 12 µm. There is no evidence of any skin sensitisation effects of any nature. The performance of studies for skin sensitisation is not considered to be required, since calcium phosphide is instable in contact with moisture. Moreover, given the acute toxicity of calcium phosphide and phosphine, it is recommended that all skin contact be avoided.

The investigation of skin sensitisation in guinea pigs with zinc phosphide shows, that the test material was classified as mild-irritant according to the classification scheme of Kay and Callandra. However, no classification is required according to the classification criteria of the EU Commission Directive 93/21 EEC.

For more details please refer to reference:

Document K, Section 3, Tier II, Annex IIA, point 5.2.6/02 [Brunt, P. (2001)].

Zinc phosphide: skin sensitisation in the guinea pig – Magnusson and Kligman maximisation method, SafePharm Laboratories Ltd., Derby, U.K., Project No. 1483/003, unpublished report, March 1, 2001]

Report: (see B.6.2.6)	Brunt, P. (2001): Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation Method, SafePharm Laboratories, Derby, U.K.; unpublished report no. 1483/003, 25.04.2001. (TOX2002-179)
Guidelines:	OECD guideline 406, Commission Directive 96/54/EC method B6 Acute Toxicity (Skin Sensitisation).
Deviations:	This study does not concern the skin sensitisation of calcium phosphide, but of zinc phosphide.
GLP:	Yes.
Acceptability:	This study is considered to be acceptable.

This study revealed no sensitising potential for zinc phosphide to guinea pig skin.

Conclusion:

“... the study for zinc phosphide revealed no skin sensitisation potential. Therefore, calcium phosphide is considered to be not a sensitising substance, too, and classification according to Commission Directive 2001/59/EC (adaptation to 67/548/EEC) is not required“ (see B.6.2).

Basing on the given data classification/labelling of the product Polytanol containing calcium phosphide is considered not necessary.

B.6.11.7 Supplementary studies for combinations of plant protection products

The plant protection product Polytanol is a ready-to-use rodenticide which should be placed into the voles' holes and only into holes which are occupied by rodents. Combinations with other plant protecting products are not envisaged.

B.6.12 Dermal absorption (Annex IIIA 7.3)

Specific experimental investigation of dermal absorption of the product was not performed since the main way of uptake is inhalative or – in case of an accident or in suicidal intention – oral. The exposition should be minimal since protective gloves are worn during production and handling of the product. In the absence of experimental data for both the active substance and the product, the dermal absorption rate for both calcium phosphide and PH_3 has to be estimated. A first estimate, based on physico-chemical properties (molecular mass and log P_{OW} , according to the ‘Guidance Document on Dermal Absorption’ of the European Commission; Doc. Sanco/222/2000 rev. 7 of 19 March 2004), would result in a default assumption of 100 % dermal absorption for both compounds. However, the same document notes that on a case by case basis it could be deviated from 100 % by expert judgement. In the case of calcium phosphide/ PH_3 , RMS concludes, that a 100 % default value would overestimate the actual dermal absorption rate, based on the following considerations: Due to the nature of the formulated product (pellets or tablets), only a minor part of the active substance, if any, is expected to come into contact with the skin. Contact with the (humid) skin surface would be expected to initiate liberation of PH_3 gas making systemic absorption highly unlikely. In previous evaluations by both the WHO (Environmental Health Criteria 73 of 1988) and the German ‘MAK-Kommission’ for the closely related substance aluminium phosphide/ PH_3 dermal absorption was stated to be negligible, in decades of approved use, no casualties or serious intoxications have been reported for operators dermally exposed to aluminium phosphide. Assuming that the oral absorption is 100 % and taking into account the acute oral study with Aluminium phosphide (Dr. Dr. W. Sterner, A. Stiglic (1977): Acute Oral Toxicity of “Aluminiumphosphid” in Rats, International Bio-Research, Hannover, Germany; unpublished report no. 0-0-51-77, 1977 (TOX2006-981) resulting in a LD_{50} (24 h) of 8.7 mg/kg bw, the dermal absorption should be very low compared to the oral absorption, since the acute dermal toxicity of aluminium phosphide (LD_{50} , 24 h) was 1520 mg/kg bw which is only 0.57 % of the acute oral LD_{50} .

Conclusion:

In conclusion, the use of a default value of 10 % is proposed, which is considered to represent a cautious and conservative approach.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

Polytanol is a gas generating product (GE) produced as pellets with a content of 180 g/kg of the pure active ingredient calcium phosphide (Ca_3P_2). The specification of the preparation is given in Annex C. The content of the active substance is calcium phosphide, techn. = 1000 g/kg and calcium phosphide, pure = 180 g/kg, thus as stated by the notifier only impurities and no formulants are to be considered into account and respective Safety Data Sheets are therefore not available.

The maximum purity of the active substance as manufactured is 280 g/kg. Thus, the impurities can vary in quantity and concentration but are considered not to be of toxicological concern.

Table B.6.13-1 Product of the notifier:

Notifier	Preparation	
Chemische Fabrik Wülfel	Polytanol	Gas generating product GE as pellets

The possibly acute oral and inhalation toxic properties are covered by the studies with the technical product Polytanol.

B.6.14 Exposure data (Annex IIIA 7.2)

Calcium phosphide (Polytanol) can be used for the control of rodents in burrows. No data on the operator exposure with calcium phosphide containing products are available. But based on measurement of PH₃ in soil and an “industrial medical check-up study” exposure assessments for operators, bystanders and workers were given by the notifier (Table B.6.14-1).

Table B.6.14-1: Overview of the submitted studies/publications and assessments

Notifier	Preparation	Chapter	Field studies and publications
Chemische Fabrik Wülfel	Polytanol	B.6.14.1	Reh, P. (2001) (TOX2002-826), see B.6.14.1.1 Koch, E. (1982) (MET2005-87, MET2006-454 [submitted to section 5, Annex IIIA, point 9.1.1.1] Konerding, J. (2004) (TOX2004-882), see B.6.14.3.1

With regard to the intended use as also assessed in the draft assessment report to aluminium phosphide for 4 different AIP-containing preparations the respective risk assessment should be considered for Polytanol, too. The respective risk assessment is supported by the submitted data of the notifier.

Thus, as it is partly approved by estimations and/or measurements it can be concluded that the risk to operators from exposure to phosphine following rodent burrow treatment is considered acceptable without the use of personal protective equipment (no PPE/RPE).

It was also demonstrated that bystander exposure and worker exposure will be acceptable.

B.6.14.1 Operator exposure

Table B.6.14-2: Description of Exposure

Intended uses	Field of use envisaged	Likely concentr. At which as will be used
Rodenticide;	Control of rodents and moles	18 % calcium phosphide

B.6.14.1.1 Estimation of operator exposure assuming personal protective equipment is not used/is used

Report: Reh, P., (2001): Polytanol - Bewertung der Exposition von Anwendern im Haus- und Kleingartenbereich, February 12, 2001. (TOX2002-826)

Presentation of the statement as given by the notifier:

Polytanol is introduced into the target animals' underground runs and tunnels by means of an applicator. This applicator is designed to be both an applicator and a dosage measuring system, which operates by means of a hand-operated crank incorporated in the handle of the applicator, on to which the product container is firmly screwed. The crank-operated dispenser feeds pellets or granules into a tube which is inserted into the underground tunnels. Pellets or granules can then be introduced one at a time into the tunnels with a simple, clear, accurate measurement of the dosage, whilst at the same time eliminating operator exposure. The applicator is a completely integrated enclosed system, from the container all the way through to the placement of the pellets or granules underground, and hence the operator has no direct contact with the pellets or granules at any time. This integrated applicator system effectively and safely prevents inhalation of calcium phosphide dust or phosphine since the user does not come into contact with the product at all. After application of Polytanol in the field, the container can be unscrewed from the applicator and firmly re-sealed with the screw cap. This operation takes no more than a few seconds, and consequently the potential for operator exposure to calcium phosphide or phosphine is effectively nil. Underground, the evolution of phosphine is not abrupt, but depends on the progressive absorption of soil moisture. Since phosphine is heavier than air, it remains within the target animals' burrows underground, and because the entrances to the burrow system are closed, the gas ultimately diffuses into the surrounding soil, with no more than trace quantities reaching the surface, well away from the respiration zone of operators or bystanders. For more details please refer to reference:

Document K, Section 5, Annex IIIA, point 9.1.1.1 (see also B.8.1.2.2) Report: Koch, E., Messungen des Diffusionsstromes von PH_3 im Boden, report no.: P/W/K 82, April 26, 1982 (MET2005-87, MET2006-454)

Phosphine has a characteristic garlic-like smell, and this can be detected at 0.02 ppm [A NOAEL in a 90 d-inhalation study in rats of 3 ppm is used for derivation of the proposed systemic AOEL for phosphine: 0.03 ppm]. This smell threshold concentration is not even comparable with the concentration needed to create a hazard to health which are typically tens or hundreds of thousands of times greater. Phosphine is not absorbed via the skin and skin contact with Polytanol is effectively prevented by the use of a sealed integrated applicator system. Moreover, even this remote risk can be further reduced by wearing of gloves.

B.6.14.1.2 Measurement of operator exposure

Justification for non-submission as given by the notifier:

Any measurement of operator exposure for the product Polytanol is not considered to be required, since this plant protection product represents a ready-to-use rodenticide which should be placed underground into target animal runs and tunnels. Polytanol is a granule with a particle size of 2 – 12 mm. The product shows a very high stability and a low content of dust (< 0.5 %).

Due to this attributes, no relevant exposure of the operator is to be expected. If in contact with moisture any phosphine will release, no relevant exposure of the operator can be expected. Phosphine is heavier than air, it remains within the target animals' burrows underground, and because the entrance to the burrow system are closed, the gas ultimately diffuses into the surrounding soil, with no more than trace quantities reaching the surface, well away from respiration zone of operators and bystanders. Than phosphine will quickly diluted in air and oxidised to phosphorous acids. For more details please refer to reference:

Document K, Section 5, Annex IIIA, point 9.1.1.1 (see also B.8.1.2.2) Report: Koch, E., Messungen des Diffusionsstromes von PH_3 im Boden, report no.: P/W/K 82, April 26, 1982 (MET2005-87, MET2006-454)

B.6.14.2 Estimation and measurement of bystander exposure

Justification for non-submission as given by the notifier:

The estimation or the measurement of bystander exposure for the product Polytanol is not considered to be required, since Polytanol should be placed underground into target animal runs and tunnels. Polytanol is a granule with a particle size of 2 – 12 mm The product shows a very high stability and a low content of dust (< 0.5 %). Due to this attributes, no relevant exposure of the operator and bystanders is to be expected. If in contact with moisture any phosphine will release, no relevant exposure of the operator and bystanders can be expected. Phosphine is heavier than air, it remains within the target animals' burrows underground, and because the entrance to the burrow system are closed, the gas ultimately diffuses into the surrounding soil, with no more than trace quantities reaching the surface, well away from respiration zone of operators and bystanders. Than phosphine will quickly diluted in air and oxidised to phosphorous acids. Particularly in view of use an applicator any quantitatively relevant exposure of bystanders to Polytanol is unlikely.

B.6.14.3 Worker exposure

B.6.14.3.1 Estimation of worker exposure assuming personal protective equipment is used

Report : Konerding, J., Arbeitsmedizinische Bescheinigung (Industrial Medicine Certification), April 5, 2004. (TOX2004-882)

Presentation of the information as given by the notifier:

Statement on medical surveillance on manufacturing plant personnel by Dr. med. Konerding (Occupational physician, Hannover, Germany):

All workers involved with the production of calcium phosphide are regularly monitored at intervals of 12 months by occupational medical inspections according to the requirements of the German national regulations on hazardous substances (Gefahrstoffverordnung). In addition to this, special comprehensive health checkups are conducted in intervals of 36 months according to recommendation no. 26 (relevant where protective respiratory devices are used) of the professional trade association (Berufsgenossenschaft). In the regular health inspections, the following parameters are assessed: differential blood count, blood glucose,

creatinine, liver status parameters, haematocrit, urine sampling. Furthermore, technical medical checkups are carried out like hearing tests, vision tests, lung function tests, x-ray investigations of the chest, and ECG and tests on olfactory nerve.

Findings:

Industrial medical check-up study

Results of industrial medical check-up study according to paragraph 28 GefStoffV to Chemische Fabrik Wülfel, Hannover, Germany, are given in Table B.6.14-3.

Table B.6.14-3: Results of industrial medical check-up study

Examination period in years	Examined persons	No. Of examinations to paragraph 28 GefStoffV	No. Of examinations to G 25	No. Of examinations to G 26.2	Examination results
16	1	14	9	8	no health considerations
10	1	10	3	4	nhc *
9	1	9	5	3	nhc *
9	1	9	1	3	nhc *
8	1	8	3*	3*	nhc *
6	2	6	2	3	nhc *
3	2	3	2	2	nhc *

*nhc (no health considerations) – under specific conditions

Conclusion:

The results of the industrial medical check-up according to paragraph 28 GefStoffV are presented in Table B.6.14-3. Nine persons in total were examined from 1987 to 2003. The examinations are performed annually. The examinations according to G25 (driving, steering and supervision abilities) and according to G26.2 (medium respiratory protection, full mask) occur every three years. The different time periods for the examined persons are the result of the duration of the respective occupational circumstances. Among the examined persons, involved in the production of Polytanol (calcium phosphide), no health impairment was detected over a period of 3 to 16 years.

B.6.14.3.2 Measurement of worker exposure

Justification for non-submission:

Not required for the reasons given in point B.6.14.3.1 above.

B.6.15 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.1	Andreev, S.B., Voevodin, A.V., Molchanova, V.A., Khotyanovich, A.V.	1958	Some results of the use of tracer techniques in the study of plant protection. 2 nd Int. Conf. Peaceful Uses Atomic Energy, 27, 1958, 85-92 not GLP, published TOX2002-165	N	-
AIIA-5.1; AIIA-5.9.4	Chan, L.T.F., Crowley, R.J., Delliou, D., Geyer, R.	1983	Phosphine analysis in post mortem specimens following ingestion of aluminium phosphide. Journal of Analytical Toxicology , 7, 1983, 165-167 not GLP, published TOX98-50056	N	-
AIIA-5.1; AIIA-5.9.4; AIIA-5.9.6	Childs, A.F., Coates, H.	1971	The toxicity of phosphorus compounds – Mellor's comprehensive treatise/inorganic and theoretical chemistry. White plains, New York, Longman (1971) Unknown not GLP, published TOX2006-170	N	-
AIIA-5.1	Curry, A.S., Price, D.E., Thyhorn, F.G.	1959	Absorption of zinc phosphide particles. Nature, 184, 1959, 642-643 not GLP, published TOX2002-163	N	-
AIIA-5.1; AIIA-5.4.2; AIIA-5.8.2	Neubert, D., Hoffmeister, I.	1960	Veränderungen im intermediären Stoffwechsel nach Einwirkung von Phosphorwasserstoff. Nannyn-Schmiedebergs's Arch. Exp. Path. U. Pharmak. , 239, 1960, 219-233 not GLP, published TOX96-52055	N	-
AIIA-5.1; AIIA-5.3; AIIA-5.6.2; AIIA-5.9.1; AIIA-5.9.2	WHO – World Health Organisation	1988	Environmental Health Criteria 73 – Phosphine and selected metal phosphides (Page 1-100). IPCS INCHEM, Environmental Health Criteria 73, WHO, Geneva, 1-100 not GLP, published TOX2005-1201	N	-
AIIA-5.2.1	Joshi, M.	1998	Acute oral toxicity of Aluminium phosphide technical in mice. 362 not GLP, unpublished TOX2006-212	Y	UPL

⁹ Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.2.1	Joshi, M.	1998	Acute oral toxicity of Aluminium phosphide technical in rats. 361 not GLP, unpublished TOX2006-211	Y	UPL
AIIA-5.2.1	Krishnakumari, M.K., Bai, K.M., Majumder, S.K.	1980	Toxicity and rodenticidal potency of zinc phosphide. Not GLP, published TOX2002-166	N	CFW
AIIA-5.2.1	Krishnakumari, M.K., Bai, K.M., Majumder, S.K.	1979	Evaluation of acute oral toxicity of zinc phosphide in Rattus norvegicus (albino). Pesticides, 13, 11, 1979, 33-35 not GLP, published TOX2002-167	N	-
AIIA-5.2.1	Leuschner, F.	1992	Acute toxicity study of Aluminium phosphide by oral administration to NMRI mice. 7129/92 GLP, unpublished TOX2005-308	N	DET
AIIA-5.2.1	Stephen, F.	2000	Acute oral toxicity of aluminium phosphide technical in rats (fixed dose method). 2565 GLP, unpublished TOX2006-210	Y	UPL
AIIA-5.2.1	Sterner, W., Chibanguza, G,	1980	Acute oral toxicity of 1 % Magnesiumphosphid in vaseline in rats. 1-4-666-79 not GLP, unpublished TOX2000-89	N	CFW
AIIA-5.2.1	Tkadlek, E., Gattermann, R.	1993	Circadian changes in susceptibility of voles and golden hamsters to acute rodenticides. Not GLP, unpublished TOX2002-170	N	CFW
AIIA-5.2.1	Nakata, K.	1993	Acute symptoms caused by 1 percent zinc phosphide pellets in the gray red-backed vole, clethrionomys rufocanus bedfordiae. Not GLP, published TOX2002-171	N	CFW
AIIA-5.2.1; AIIA-5.3.1; AIIA-5.3.2; AIIA-7.1.1	Venugopala Rao, K.	1999	Acute oral toxicity study with Polytanol in Wistar rats. 2739/99 GLP, unpublished TOX2000-105	Y	CFW

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.2.2; AIII-7.1.2	Dickhaus, S., Heisler, E.	1987	Akute Toxizitätsprüfung von der Substanz Aluminiumphosphid nach dermalen Applikation an der Ratte (OECD-Guidelines no. 404) (Acute toxicological study on compound Aluminiumphosphid after dermal application to the rat). 1-4-142-87 GLP, unpublished TOX2000-93	N	DET
AIIA-5.2.2	Dickhaus, S., Heisler, E.	1980	Akute Toxizitätsprüfung von der Substanz „Zinkphosphid“ nach dermalen Applikation an der skarifizierten Haut der Ratte; Translation: Acute toxicity test of the substance zinc phosphide after dermal application to the skarified skin of rats. 1-4-258a-80 not GLP, unpublished TOX2002-173	Y	CFW
AIIA-5.2.2	Dickhaus, S., Heisler, E.	1980	Akute Toxizitätsprüfung von der Substanz „Zinkphosphid“ nach dermalen Applikation an der Ratte. 1-4-258-80 not GLP, unpublished TOX96-52062	Y	CFW
AIIA-5.2.2	Dickhaus, S., Heisler, E.	1980	Akute Toxizitätsprüfung von der Substanz „Zinkphosphid“ nach dermalen Applikation an der Ratte. Translation: Acute toxicity test of the substance zinc phosphide after dermal application to the rat. 1-4-258-80 not GLP, unpublished TOX2002-172	Y	CFW
AIIA-5.2.2	Joshi, M.	1998	Acute dermal toxicity of Aluminium phosphide technical in rats. 363 not GLP, unpublished TOX2006-214	Y	UPL
AIIA-5.2.2	Köhler, U.	2004	Calcium phosphide: Acute percutaneous toxicity. GLP, unpublished TOX2000-110	Y	CFW
AIIA-5.2.2	Stephen, F.	2000	Acute dermal toxicity of Aluminium phosphide technical in rats. 2566 GLP, unpublished TOX2006-213	Y	UPL

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.2.3	Muthu, M., Krishnakumari, M.K., Muralidhara, Majumder, S.K.	1980	A study on the acute inhalation toxicity of phosphine to albino rats. Bulletin of environmental contamination and toxicology (1980) Vol. 24 pp.404-410 Unknown not GLP, published TOX2006-171	N	-
AIIA-5.2.3; AIIIA-7.1.3	Newton, P.E.	1989	An acute inhalation toxicity study of phosphine (PH ₃) in the rat. PROJECT NO.: 87-8029 not GLP, unpublished TOX97-51198	N	CAS
AIIA-5.2.3; AIIA-5.3; AIIA-5.6.1; AIIA-5.6.2; AIIIA-7.1.3	Newton, P.E., Schroeder, R.E., Sullivan, J.B. et.al.	1993	Inhalation toxicity of phosphine in the rat: Acute, subchronic, and developmental. Inhalation Toxicology, 5, 2, 1993, 223-239 Not GLP, published TOX2002-824	N	-
AIIA-5.2.3	Roy, B.C.	1998	Acute inhalation toxicity of Aluminium phosphide technical in rats. 366 not GLP, unpublished TOX2006-215	Y	UPL
AIIA-5.2.3; AIIIA-7.1.3	Shimizu, Y., Ogawa, Y., Tokiwa, K.	1982	Acute inhalation toxicity testing of hydrogen phosphide in rats. NRI 82-7489 not GLP, unpublished TOX2005-280	N	DET
AIIA-5.2.3; AIIA-5.3.3; AIIIA-7.1.3	Venugopala Rao, K.	2001	Acute inhalation toxicity study with Polytanol in Wistar rats. 3078/00 GLP, unpublished TOX2002-822	Y	CFW
AIIA-5.2.3	Waritz, R.S., Brown, R.M.	1975	Acute and subacute inhalation toxicities of phosphine, phenylphosphine and triphenylphosphine. Am. Ind. Hyg. Assoc. J., 36, 1975, 452-458 not GLP, published TOX2002-828	N	-
AIIA-5.2.3	Waritz, R.S., Brown, R.M.	1975	Acute and subacute inhalation toxicities of phosphine, phenylphosphine and triphenylphosphine. Am. Ind. Hyg. Assoc., 36, 1975, 452-458 not GLP, published TOX2002-176	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.2.4	Brunt, P.	2001	Zinc phosphide: Acute dermal irritation in the rabbit. 1483/001 GLP, unpublished TOX2005-168	Y	CFW
AIIA-5.2.4; AIIIA-7.1.4	Dickhaus, S., Heisler, E.	1987	Prüfung von Aluminiumphosphid auf primäre Hautreizwirkung an der intakten Haut des Kaninchens (OECD-Guidelines no. 406). (Irritant effects of Aluminiumphosphid on intact skin of Rabbits). 1-3-183-87 GLP, unpublished TOX2000-94	N	DET
AIIA-5.2.4	Joshi, M.	1998	Primary skin irritation of Aluminium phosphide technical in rabbits. 364 not GLP, unpublished TOX2006-216	Y	UPL
AIIA-5.2.4	Köhler, U.	2004	Calcium phosphide: Skin irritation. Not GLP, unpublished TOX2005-165	N	CFW
AIIA-5.2.5	Brunt, P.	2001	Zinc phosphide: Acute eye irritation in the rabbit. 1483/002 GLP, unpublished TOX2005-171	Y	CFW
AIIA-5.2.5; AIIIA-7.1.5	Dickhaus, S., Heisler, E.	1987	Augenreiztest am Kaninchen mit Aluminiumphosphid nach OECD no. 405. (Irritant effects of Aluminiumphosphid on rabbit eye). 1-3-184-87 not GLP, unpublished TOX2000-95	N	DET
AIIA-5.2.5	Köhler, U.	2004	Calcium phosphide: Eye irritation. Not GLP, unpublished TOX2005-170	N	CFW
AIIA-5.2.6; AIIIA-7.1.6	Brunt, P.	2001	Zinc phosphide: Skin sensitisation in the guinea pig – Magnusson and Kligman maximisation method. 1483/003 GLP, unpublished TOX2002-179	Y	CFW
AIIA-5.2.6	Köhler, U.	2004	Calcium phosphide: Skin sensitisation. Not GLP, unpublished TOX2005-172	N	CFW

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.3; AIIA-5.4.1; AIIA-5.6.2; AIIA-5.8.2	Potter, W.T., Rong, S., Griffith, J., White, J., Garry, V.F.	1991	Phosphine-mediated Heinz body formation and hemoglobin oxidation in human erythrocytes. Toxicology Letters, 57, 1991, 37-45 Not GLP, published TOX2000-113	N	-
AIIA-5.3.2; AIIA-5.4.2	Barbosa, A., Rosinova, E., Dempsey, J., Bonin, A.M.	1994	Determination of genotoxic and other effects in mice following short term repeated-dose and subchronic inhalation exposure to phosphine. Environmental and Molecular Mutagenesis, 24, 1994, 81-88 not GLP, published TOX97-50676	N	-
AIIA-5.3.2; AIIA-5.6.2	Jokote, C.	1904	Experimentelle Studien über den Einfluß technisch und hygienisch wichtiger Gase und Dämpfe auf den Organismus, Teil XI. Studien über Phosphorwasserstoff. Arch. Für Hyg., 49/50, 275-306 not GLP, published TOX2005-1198	N	-
AIIA-5.3.2; AIIA-5.5; AIIA-5.6.2	Kietzmann, M.	2004	Statement of concerning toxicological studies with calcium phosphide (Polytanol). Not GLP, unpublished TOX2005-178	Y	CFW
AIIA-5.3.2; AIIA-5.4.2	Kligerman, A.D., Bishop, J.B., Erexson, G.L. et. Al.	1994	Cytogenetic and germ cell effects of phosphine inhalation by rodents. II: Subacute exposures to rats and mice. Environmental and Molecular Mutagenesis, 24, 1994, 301-306 not GLP, published TOX2002-830	N	-
AIIA-5.3.2	Morgan, D. L., Moorman, M. P., Elwell, M. R., et al.	1995	Inhalation toxicity of Phosphine for Fischer 344 rats and B6C3F1 mice. Inhalation Toxicology, 7, 1995, 225-238 not GLP, published TOX2002-181	N	-
AIIA-5.3.2	Muktha Bai, K., Krishnakumari, M.K., Ramesh, H.T., et al.;	1980	Short term toxicity study of zinc phosphide in albino rats (rattus norvegicus). Indian Journal of Exp., 18, 1980, 854-857 not GLP, published TOX2005-175	N	-
AIIA-5.3.2; AIIA-5.6.2	Okolie, N.P., Aligbe, J.U., Osakue, E.E.	2004	Phostoxin-induced biochemical and patho-morphological changes in rabbits. Indian J. Exp. Biol., 42, 11, 2004, 1096-1099 not GLP, published TOX2005-1200	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.3.2	Schnellhardt, M., Gebhardt, M., Bräter, M., et al.;	1985	Study on the subchronical toxicity of Aluminium phosphide – 90-day-test on growing wistar rats. Not GLP, unpublished TOX2005-282	N	DET
AIIA-5.3.3	Newton, P.E.	1990	An thirteen week inhalation toxicity study of phosphine (PH ₃) in the rat. 87-8030 GLP, unpublished TOX2001-684	Y	PSA
AIIA-5.4.1	Araki, A., Noguchi, T., Kato, F., Matsushima, T.	1994	Improved method for mutagenicity testing of gaseous compounds by using a gas sampling bag. Mutation Research, 307, 1994, 335-344 not GLP, published TOX2002-182	N	-
AIIA-5.4.1; AIIA-5.9.1	Garry, V.F., Griffith, J., Danzl, Th.J., Nelson, R.L., Whorton, E.B., Krueger, L.A., Cervenka, J.	1989	Human genotoxicity: Pesticide applicators and phosphine. Science, 246, 1989, 251-255 not GLP, published TOX2000-114	N	-
AIIA-5.4.1	Leuschner, F.	1992	Phosphine: Mutagenicity study in mammalian cells (V79) in vitro – HGPRT Test. 6990/91 GLP, unpublished TOX2005-284	N	DET
AIIA-5.4.1	Rajwani, L.S.	2000	Salmonella typhimurium reverse mutation assay of Aluminium phosphide technical. 2567 GLP, unpublished TOX2006-220	Y	UPL
AIIA-5.4.1	SanSebastian, J.R.	1990	Structural chromosome aberration in Chinese hamster ovary (CHO) cells induced by hydrogen phosphide (PH ₃). (in vitro). PH 320-DA-001-89 GLP, unpublished TOX2001-686	Y	PSA
AIIA-5.4.1	Stankowski, L.F.	1990	Ames/salmonella plate incorporation assay on hydrogen phosphide (PH ₃). PH 301-DA-001-89 GLP, unpublished TOX2001-685	Y	DET

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.4.1	Sutou, S., Yamamoto, K., Shirakawa, H.	1982	In vitro microbial mutagenicity testing of hydrogen phosphide. 82-7492 not GLP, unpublished TOX2005-283	N	DET
AIIA-5.4.2	Guna Sherlin, D.M.	1998	Chromosomal aberration test of Aluminium phosphide technical in mice. 367 not GLP, unpublished TOX2006-222	Y	UPL
AIIA-5.4.2	Guna Sherlin, D.M	1998	Micronucleus test of Aluminium phosphide technical in mice. 368 not GLP, unpublished TOX2006-221	Y	UPL
AIIA-5.4.2; AIIA-5.4.3; AIIA-5.6.1	Kligerman, A.D., Bishop, J.B., Erexson, G. L. et al.	1994	Cytogenetic and germ cell effects of phosphine inhalation by rodents. II: Subacute exposures to rats and mice. Environmental and Molecular Mutagenesis, 24, 1994, 301-308 not GLP, published TOX2002-185	N	-
AIIA-5.4.2	Kligerman, A.D., Bryant, M.F., Doerr, C.L., et al.	1994	Cytogenetic effects of phosphine inhalation by rodents. I: Acute 6-hour exposure of mice. Environmental and Molecular Mutagenesis, 23, 1994, 186-189 not GLP, published TOX2002-184	N	-
AIIA-5.4.2	Kligerman, A.D., Bryant, M.F., Doerr, C.L., Erexson, G.L., Kwany- uen, P., McGee, J.K.	1994	Cytogenetic effects of phosphine inhalation by rodents. I: Acute 6-hour exposure of mice. Environmental and Molecular Mutagenesis , 23, 1994, 186-189 not GLP, published TOX97-50677	N	-
AIIA-5.4.2	McKeon, M.E.	1993	Genotoxicity test on Phosphine in the in vivo/in vitro assay for unscheduled DNA synthesis in rat primary hepatocyte cultures at two timepoints. A0040-0-494 GLP, unpublished TOX2005-285	Y	DET
AIIA-5.4.2; AIIA-5.6.1	Rajesh Sundar, S.	1999	Dominant lethal test of Aluminium phosphide technical in mice. 370 not GLP, unpublished TOX2006-224	Y	UPL

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.5	Newton, P.E., Hilaski, J., Banas, D.A. et al.	1999	A 2-year inhalation study of phosphine in rats. Inhalation Toxicology, 11, 1999, 693-708 not GLP, published TOX2002-189	N	-
AIIA-5.5	Newton, P.E.	1998	2-year combined inhalation chronic toxicity and oncogenicity study of phosphine in rats. 750-001 ! 182-007 GLP, unpublished TOX2000-98	Y	DET
AIIA-5.6.1	Köhler, U.	2004	Calcium phosphide: Two generation reproduc- tive in the rat. Not GLP, unpublished TOX2005-188	N	CFW
AIIA-5.6.2	Anonymous	1991	Poisons Information Monograph 865 – Phosphine. IPCS INCHEM not GLP, published TOX2005-1197	N	-
AIIA-5.6.2	Neurath, G.	2005	Phosphine/metal phosphides: Justification for non-submission of a developmental toxicity/teratogenicity study in the rabbit with phosphine – Expert Statement. 149288-A2-050602-01 not GLP, unpublished TOX2005-1127	N	CFW
AIIA-5.6.2	Schroeder, R.E.	1989	An inhalation developmental toxicity study of phosphine (PH ₃) in rats. 89-3413 GLP, unpublished TOX2001-687	Y	PSA
AIIA-5.6.10	Müller, W.	1940	Über Phosphorwasserstoffvergiftung (Tierver- suche). I. Mitteilung: Akute und subakute Vergiftung not GLP, published TOX2005-1199	N	DET
AIIA-5.6.10	Newton, P.E. et al.	1993	Inhalation toxicity of phosphine in the rat: Acute, subchronic, and developmental. not GLP, published TOX2002-824	N	-
AIIA-5.7; AIIA-5.8.2	Schaefer, G.J.	1998	90-day inhalation neurotoxicity study of phosphine in rats Vol. 1 to 5. 750-003 GLP, unpublished TOX2006-176	Y	CAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.7	Schaefer, G.J.	1998	Acute neurotoxicity study of phosphide in rats. (delayed neurotoxicity following acute exposure). 750-002 GLP, unpublished TOX2000-97	Y	DET
AIIA-5.7	Schaefer, G.J., Newton, P. E., Gruebbel, M.M. et al.	1998	Acute and subchronic inhalation neurotoxicity of phosphine in the rat. Inhalation Toxicology, 10, 1998, 293-320 not GLP, published TOX2002-190	N	-
AIIA-5.9.1; AIIA-5.9.5	Anonymous	1999	Emergency first aid treatment guide for zinc phosphide. EPA, 1999 not GLP, published TOX2002-209	N	-
AIIA-5.9.1	Asher, P.P.	2005	Report on health status of factory workers exposed during manufacturing of Aluminium phosphide. Not GLP, unpublished TOX2006-225	Y	UPL
AIIA-5.9.1; AIIA-5.9.3; AIIIA-7.2.1.2	Barbosa, A., Bonin, A.M.	1994	Evaluation of phosphine genotoxicity at occu- pational levels of exposure in New South Wales, Australia. Occup.-Environ.-Med. , 51, 10, 1994, 700-705 not GLP, published TOX97-50675	N	-
AIIA-5.9.1	Guth, E.	2003	Occupational Health Care for Employees under Hydrogen Phosphide (PH ₃) Exposition. not GLP, unpublished TOX2004-2205	N	DET
AIIA-5.9.1	Henschler	1987	Gesundheitsschädliche Arbeitsstoffe – Toxi- kologisch-arbeitsmedizinische Begründung von MAK-Werten. VCH; 1-12 Lieferung/1986/87. Phosphorwasserstoff. (Translation: Industrial substances dangerous to health – Toxicological occupational health justification of MAK values). not GLP, unpublished TOX97-50342	Y	CFW
AIIA-5.9.1	Köhler, U.	2004	Arbeitsmedizinische Vorsorgeuntersuchungen des Betriebspersonals – Direkte Beobachtun- gen –Industrial medical check-up of factory personnel – direct observations. Translation: Industrial medical check-up of factory person- nel – direct observations. not GLP, unpublished TOX2005-194	N	CFW

WARNING: This document forms part of an EC evaluation data package and must not be released for public use without the express written permission of the Commission. Registration must not be granted on the basis of this document.

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.9.1; AIIA-5.9.2; AIIA-7.2.3.1	Konerding, J.	2004	Arbeitsmedizinische Bescheinigung. Translati- on: Industrial Medicine Certification. Not GLP, unpublished TOX2004-882	N	CFW
AIIA-5.9.1	Misra, U., Bhargava, S., Nag, D., Kidwai, M., Lal M.	1988	Occupational phosphine exposure in indian workers. Toxicology letters, 42, 257-263 Unknown not GLP, published TOX2006-182	N	-
AIIA-5.9.2; AIIA-5.9.6	Anonymous	1999	Niosh Alert – Preventing phosphine poisoning and explosions during fumigation. National Institute for Occupational Safety and Health, Cincinnati, USA, DHHH (NIOSH) publication No. 99-126 not GLP, published TOX2006-226	N	-
AIIA-5.9.2; AIIA-5.9.4; AIIA-5.9.5; AIIA-5.9.6	Balali-mood, M.	1991	Phosphine – International programme on chemical safety, poisons information monograph 865. International Programme on Chemical Safety (IPCS) PIM 865 not GLP, published TOX2006-185	N	-
AIIA-5.9.2	Brockstedt, M.	1991	Inquiry with regard to the active substance zinc phosphide – Engl. Transl. Of German doc.: Anfragen zum Wirkstoff Zinkphosphid not GLP, unpublished TOX2006-688	Y	CFW
AIIA-5.9.2; AIIA-5.9.4	Chugh, S.N. et al.;	1991	Incidence & outcome of aluminium phosphide poisoning in a hospital study. Indian J Med Res, 232-235 not GLP, published TOX2005-305	N	-
AIIA-5.9.2	Chugh, S.N., Ram, S., Sharma, A., Arora, B.B. et al.	1989	Adrenocortical involvement in aluminium phosphide poisoning. Indian J Med Res, 90, 289-294 not GLP, published TOX2007-169	N	-
AIIA-5.9.2	Chugh, S.N., Sant Ram, Chugh, K., Malhotra, K.C.	1989	Spot diagnosis of aluminium phosphide ingestion: An application of a simple test. J. Assoc. Physicians India, 37, 219 not GLP, published TOX2007-168	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.9.2	Feldstein, A., Heumann, M., Barnett, M.	1991	Fumigant intoxication during transport of grain by railroad. Journal of Occupational Medicine (1991) Vol.33, pp. 64-65 Unknown not GLP, published TOX2006-186	N	-
AIIA-5.9.2	Gellner, R.	1991	Inquiry with regard to the pesticide active substance - Engl. transl. of German doc.: Anfrage zu Pflanzenschutzmittelwirkstoff Zinkphosphid not GLP, unpublished TOX2006-696	Y	-
AIIA-5.9.2	Glass, A.	1956	Account of suspected phosphine poisoning in a submarine. Journal Royal Navy Medical Services (1956) Vol. 42, pp. 185-187 Unknown not GLP, published TOX2006-187	N	-
AIIA-5.9.2	Gosselin, R.E.; Smith, R.P.; Hodge, H.C. and Braddock, J.E.	1984	Zinc phosphide. Clinical toxicology of commercial products. Fifth edition, Williams & Wilkins – Baltimore Hong Kong London Sydney, 1984, II-120 not GLP, published TOX2002-199	N	-
AIIA-5.9.2	Gupta, S., Sushil, K., Ahlawat, K.	1995	Aluminium phosphide poisoning – a review. Clinical Toxicology, 33, 1, 1995, 19-24 Unknown not GLP, published TOX2006-188	N	-
AIIA-5.9.2	Harger, R.N., Spolyar, L.W.	1958	Toxicity of phosphine, with a possible fatality from this poison. Arch. Ind. Health, 18, 497-504 not GLP, published TOX2007-171	N	-
AIIA-5.9.2	Hayes, W.J.	1982	Pesticides studied in man. (Organic phosphorus pesticides, clinical considerations, potentiation and antagonism, chlorfenvinphos.). Pesticides studied in man. Williams & Wilkins; Baltimore/London, , 1982, 301-318 132AX-493-089 not GLP, published TOX96-52228	N	-
AIIA-5.9.2	Khosla, S.N., Nand, N., Khosla, P.	1988	Aluminium phosphide poisoning. Med. Hyg., 91, 196-198 not GLP, published TOX2007-170	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.9.2	Khosla, S.N., Handa, R., Khospla, P.	1992	Aluminium phosphide poisoning. Tropical Doctor (1992) Vol.22, pp. 155-157 Unknown not GLP, published TOX2006-189	N	-
AIIA-5.9.2	Kowaleswski, S., Smend, J.	1991	Inquiry with regard to the pesticide active substance (Engl. Transl.) – Anfrage zu Pflan- zenschutzmittelwirkstoff Zinkphosphid. Not GLP, unpublished TOX2006-25	Y	CFW
AIIA-5.2.9	Martens, F.	1991	Inquiry with regard to the pesticide active substance – Engl. Transl. Of German doc.: Anfrage zu Pflanzenschutzmittelwirkstoff Zinkphosphid not GLP, unpublished TOX2006-692	Y	CFW
AIIA-5.9.2	Moll, H.	1991	Inquiry with regard to the pesticide active substance - Engl. transl. of German doc.: Anfrage zu Pflanzenschutzmittelwirkstoff Zinkphosphid not GLP, unpublished TOX2006-697	Y	-
AIIA-5.9.2	Rodenberg, H.D., Chang, C.C. and Watson, W.A.	1989	Zinc phosphide ingestion: A case report and review. Vet. Hum. Toxicol., 31, 6, 1989, 559-562 not GLP, published TOX2002-200	N	-
AIIA-5.9.2	Sarma, P.S.A., Narula, J.	1996	Acute pancreatitis due to Zinc phosphide ingestion. Postgraduate Med. J., 72, 1996, 237-238 not GLP, published TOX2006-26	N	-
AIIA-5.9.2	Siwach, S.B., Yadav, D.R., Arora, B., Dalal, S.	1988	Acute aluminium phosphide poisoning, an epidemiological, clinical, and histopathological study. Journal Association of Physicians India (1988) Vol. 36, pp. 594-6 Unknown not GLP, published TOX2006-191	N	-
AIIA-5.9.2	Stephenson, J.B.P.	1967	Zinc phosphide poisoning not GLP, published TOX96-52073	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.9.2	Wilson, R., Lovejoy, F.H., Jaeger, R.J., Landrigan, P.L.	1980	Acute phosphine poisoning aboard a grain freighter. Epidemiologic, clinical and pathological findings. JAMA, 244, 148-150 not GLP, published TOX2007-172	N	-
AIIA-5.9.2	Zipf, K. E., Arndt, T.; Heintz, R.	1967	Clinical observation of a case of Phostoxin poisoning. Springer Verlag not GLP, published TOX2005-287	N	-
AIIA-5.9.3	Amr, M., Allam, M., Osmaan, A.L. et al.	1993	Neurobehavioral changes among workers in some chemical industries in Egypt. Environmental Research, 63, 1993, 295-300 not GLP, published TOX2002-201	N	-
AIIA-5.9.3	Amr, M. M.; et al.;	1997	Neuropsychiatric syndromes and occupational exposure to Zinc phosphide in Egypt. Environ. Res., 73, 1997, 200-206 not GLP, published TOX2006-27	N	-
AIIA-5.9.3	Burgess, J.L., Pappas, G.P., Robertson, W.O.	1997	Hazardous material incidents: The Washington poison center experience and approach to exposure assessment. JOEM, 8, 39, 1997, 760-766 not GLP, published TOX2005-195	N	-
AIIA-5.9.3	Burgess, J.L., Morrissey, B., Robertson, W.O.	1998	Fumigant related illnesses: Washington State's five year experience. J. Toxicol. Clin. Toxicol., 36, 1998, 465 not GLP, published TOX2002-203	N	-
AIIA-5.9.3	Garry, V.F., et al.;	1992	Chromosome rearrangements in fumigant applicators: Possible relationship to non-Hodgkins lymphoma risk. Cancer Epidemiol. Bio. Prev., 1, 1992, 287-291 not GLP, published TOX2006-30	N	-
AIIA-5.9.3	Garry, V.F., et al.;	1989	Human genotoxicity: Pesticide applicators and phosphine. Science, 246, 1989, 251-255 not GLP, published TOX2006-28	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.9.3	Halloran, R.O., Reich, G.A.	1970	Blindness and gastrointestinal tract symptoms after exposure to pesticides. JAMA, 214, 5, 1970, 920 not GLP, published TOX2002-202	N	-
AIIA-5.9.3	Sorensen, K.J., et al.;	1996	The effect of phosphine, smoking and age on stable chromosome aberration frequencies in agricultural workers. Environ. Mol. Mutagen. Abstr., 27, 1996, 64 not GLP, published TOX2006-29	N	-
AIIA-5.9.3	Tucker, J.D., et al.;	2003	Multi-endpoint biological monitoring of phosphine workers. Mutat. Res., 536, 2003, 7-14 not GLP, published TOX2006-31	N	-
AIIA-5.9.4; AIIA-5.9.5; AIIA-5.9.6	Backhaus, A.	1997	Medizinisches Merkblatt für den Arbeitsschutz - Unfälle mit Polytanol - Medical information for worker protection - Accidents with Polytanol. not GLP, unpublished TOX2000-122	N	CFW
AIIA-5.9.4	Guale, F.G., Stair, E.L., Johnson, B.W. et al.	1994	Laboratory diagnosis of zinc phosphide poisoning. Vet. Human Toxicol., 36, 1994, 517-519 not GLP, published TOX2002-205	N	-
AIIA-5.9.4; AIIA-5.10	Klimmer, O.R.	1971	Phosphorwasserstoff und Phosphide in Pflan- zenschutz- und Schädlingsbekämpfungsmittel- Abriss einer Toxicologie und Therapie von Vergiftungen. not GLP, published TOX2005-197	N	-
AIIA-5.9.4	Köhler, U.	2004	Calcium phosphide: Poisoning diagnosis. not GLP, unpublished TOX2005-198	N	CFW
AIIA-5.9.4	Reigart, J.R. and Roberts, J.R.	1999	Recognition and management of pesticide poisonings. EPA Manual, fifth edition, chapter 17, 1999, 169-182 not GLP, published TOX2002-207	N	-
AIIA-5.9.5	Chopra, J.S., Karla, O.P., Malik, B.S. et al.	1988	Aluminium phosphide poisoning: a prospective study of 16 cases in one year. Postgrad. Med. J., 62, 1113-1115 not GLP, published TOX2007-173	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIIA-5.9.5	Köhler, U.	2004	Prescribed treatment - Immediate measures. not GLP, unpublished TOX2005-199	N	CFW
AIIA-5.9.5	Shadnia, S., Rahimi, M., Pajoumand, A., Rasouli, M.H., Abdollahi, M.	2005	Successful treatment of acute aluminium phosphide poisoning: possible benefit of coconut oil. Human & Experimental Toxicology (2005) Vol.24, pp. 215-218 Unknown not GLP, published TOX2006-192	N	-
AIIA-5.9.5	Suman, R.L., Savani, M.	1999	Pleural effusion - A rare complication of Aluminium phosphide poisoning. Indian Pediatrics, 36, pp 1161-1163 not GLP, published TOX2006-227	N	-
AIIA-5.9.5	Weller, D.	1982	Toxicology of hydrogen Phosphide (Phosphine) - Therapy of poisoning. not GLP, published TOX2005-306	N	-
AIIA-5.9.6	Misra, U.K., Tripathi, A.K., Pandey, R. Bhargwa,	1988	Acute Phosphine poisoning following ingestion of Aluminium phosphide. Human Toxicol., 343-345 not GLP, published TOX2005-307	N	-
AIIA-5.9.6	Singh, S., Dila- wari, J.B., Yashist, R. et al.	1985	Aluminium phosphide ingestion. Br. Med. J., 290, 1110 not GLP, published TOX2007-177	N	-
AIIA-5.10; AIIIA-7.2.1.1	Reh, P.	2001	Polytanol - Bewertung der Exposition von Anwendern im Haus- und Kleingartenbereich. not GLP, unpublished TOX2002-826	N	CFW
AIIIA-7.1.1	Sterner, W., Stiglic, A.	1977	Acute oral toxicity of "Aluminiumphosphid" in rats. 0-0-51-77 not GLP, unpublished TOX2006-981	N	DET
AIIIA-7.2.1.2	Tucker, J.D. Moore, D.H. Ramsay, M.J., et al.;	2003	Multi-endpoint biological monitoring of Phosphine workers. Mutation Research, 536, 2003, 7-14 not GLP, published TOX2005-336	N	-
AIIIA-7.4	Köhler, U.	2004	Polytanol: Available toxicological data for each formulant. not GLP, unpublished TOX2005-211	N	CFW

WARNING: This document forms part of an Evaluation data package and is not to be used for the purpose of registration. Registration must not be granted on the basis of this document.

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁹
AIHA-7.4	Köhler, U.	2004	Polytanol: Notification and safety data sheet submitted in the context of Directive 67/548/EEC and Commission Directive 91/155/EEC for each formulant. not GLP, unpublished TOX2005-210	N	CFW

Codes of owner

CAS: CASA BERNADO LTDA
 CFW: Chemische Fabrik Wülfel
 DET: Detia Freyberg GmbH
 PSA: Prosanitas GmbH
 UPL: United Phosphorus Ltd.