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A Study on the Acute Inhalation Toxicity of Phosphine to Albino Rats

M. Muthu, M. K. Krishnakumari, Muralidhara, and S. K. Majumder
Central Food Technological Research Institute, Mysore 570013, India

Phosphine gas generated from aluminium phosphide preparations (Phostoxin, Delicia, Celphos, Phosfume, Quickphos, etc.) is being extensively employed as a general fumigant to control stored product insects and rodents all over the world.

Registration of aluminium phosphide preparations entails submission of relevant toxicological data to the Government bodies with a view to ensuring customer/consumer safety in practical use. The present investigation was undertaken to provide inhalation toxicity data for two such aluminium phosphide preparations requiring registration. Little published information is available on acute inhalation toxicity parameters like the median lethal dose and the LD_{95} of phosphine to rats. According to the FAO/WHO Expert Committee on Pesticides in Agriculture and Pesticide Residues (1965) there are no data available on the acute LD_{50} of aluminium Phosphide. An LC_{50} of 0.44 μM per litre at an exposure of 4 hr has been determined for the male rat in inhalation toxicity tests (WARITZ and BROWN 1975).

MATERIALS AND METHODS

Animals: Adult female albino rats (Rattus norvegicus albinicus) of the CFT-Wistar strain (175 to 225 g) were used for the tests. The animals were fed on basal diet and had free access to water.

Inhalation Exposure: An insulated aluminium panelled gas-tight atmospheric vault which maintained whatever temperature was there before closing the door, within close limits, was the test chamber. The volume was 5,943 litres. It was provided with a push-fit glass-panelled door which was sealed to the rubber gasketed door

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frame with cellophane tape. CLARKE (1977) has advocated chambers as large as 500 litres per every Kg body weight of exposed animal per hour of exposure, to overcome the problem of carbon dioxide accumulation. Based on the above criterion it was found that the chamber used by us would suffice approximately for an exposure period of 9-11 hours. In our studies rats kept inside for 24 hours in the closed chamber remained healthy and normal.

Six adult rats were exposed per dose in a 22.9 cm X 30.5 cm X 38.1 cm wire mesh cage and provided with water. Aluminium phosphide (AlP) pellets (approx. 0.6 g yielding 0.2 g PH_3) were dropped into a (250 ml) beaker containing distilled water located on a wooden stool in the centre of the chamber and the door sealed immediately. There was a quick evolution of phosphine as could be seen from the bubbles arising in the beaker. The water retains ammonia and carbon dioxide evolved along with phosphine and hence will not interfere with the toxicity test for phosphine. However, one of the aluminium phosphide preparations tested did not contain ammonium carbamate responsible for generating ammonia and carbon dioxide.

By varying the number of AlP pellets and exposure times different concentration-time products were obtained.

Analysis of Atmosphere: A gas sampling polyethylene tube (5 mm i.d.) was provided with one end located near the animal cage and the other terminating outside the chamber for estimating the gas concentrations. The "Phosphine Detector Tube" developed by MUTHU and MAJUMDER (1973) was employed for the analysis. Concentrations were determined at the end of 0.5 hrs. and every hour thereafter during the exposure period. The concentrations (mg/L) were plotted against exposure (hrs) and the concentration-time curves drawn. The integrated concentration-time products (CT) were computed by measuring the area under the curve (BROWN 1959).

Observations: After exposure the animals were retrieved from the chamber and the symptoms and mortality recorded. Survivors were transferred to individual cages and observed for four weeks. The basal diet and water were provided to the

animals during this period, ad libitum. Delayed mortality was recorded before arriving at the final mortality figures. Weekly body weights were also recorded. The survivors were autopsied at the end of the fourth week. The weights of liver, lung, kidney, heart, spleen, ovary and adrenal were recorded and the tissues processed for histopathological examination.

The mortality data were statistically analysed by the method of LITCHFIELD and WILCOXON (1949). The approximate mean concentrations were arrived at by dividing the integrated CT products by the exposure times (TABLES 1 and 2) for each test

TABLE 1

Range of concentrations of phosphine and exposure times tested on adult female albino rats

Sample	No. of AlP pellets	Integrated c.t. product mg/h/L	Calculated mean concentration		Exposure time (hrs)	Mortality (%)
			mg/L	ppm		
Control (No Gas)	0	0	0	0	24	0
A	2	0.190	0.03	20	6	33.3
	4	0.242	0.06	40	4	66.6
	2	0.352	0.04	27	8	83.3
	4	0.395	0.06	40	6	100
B	2	0.284	0.05	33	6	16.6
	4	0.366	0.09	60	4	33.3
	2	0.410	0.05	33	8	83.3

RESULTS

Inhalation Exposures: The range of dosages tried, integrated concentration time products, mean concentrations and related exposure hours and mortality are shown in TABLE 1. In general symptoms observed during exposure

TABLE 2

Acute LC_{50} and LC_{95} of phosphine gas (generated from aluminium phosphide pellets) to adult female albino rats

Product & chamber temperature °C	Parameter	Integrated Concentration time product mg/h/L	95% Confidence limits		Slope (95% confidence limits)	Mean concentration		Exposure hr
			Upper	Lower		mg/L	ppm	
A 27 ± 2°C	LC_{50}	0.22	0.27	0.18	1.46 (1.08-1.97)	0.042	28	5.2
	LC_{95}	0.42	0.67	0.26		0.068	45	6.2
B 26.1 ± 1.5°C	LC_{50}	0.36	0.42	0.31	1.21 (1.01-1.44)	0.05	33.3	7.4
	LC_{95}	0.49	0.69	0.35		0.05	33.3	8.8

were polyuria and dyspnea. The affected rats appeared listless and gasped heavily. Some lost muscular co-ordination resulting in loss of balance. Paralysis was common and the animals died suddenly. In a few cases animals found gasping, survived. Most of the mortality (75%) occurred during exposure. Around 20% died within 1-2 hours during post-exposure. Late mortality was rare (5%).

Food Intake: Survivors did not consume food for a period of nearly 48 hours though they drank water. The food consumption among survivors normalised after 3-4 days.

Body Weights and Organ Weights: There was a general decrease in body weights at the end of the first week due probably to decreased food intake, whereafter the weights reverted to the normal range. There was no significant difference in the weight gain among the rats exposed to different dosages tested. No marked morphological changes were observed in the liver, kidney, lung, heart and spleen, but the relative weights of the lungs increased slightly with increments in the dosage.

Histopathology: Lungs suffered the most damage. The symptoms varied from mild to severe cellular infiltration around the bronchioles. Some lungs were mildly edematous.

The LC_{50} and LC_{95}: The data are shown in TABLE 2. The LC_{50} values of PH_3 ranged from 0.22 mg.hr /L (27°C) to 0.36 mg.hr /L (26.1°C) with related exposure periods of 5.2 to 7.4 hours respectively for the products A and B. The LC_{95} for products A and B were 0.42 and 0.49 mg.hr /L with exposure periods of 6.2 and 8.8 hr respectively.

DISCUSSION

LOVENTHAL (1949) has stated that subacute poisoning produced degenerative changes of the ganglion cells indicating that PH_3 must be considered as a central nervous system poison. The brain and liver lesions appeared to be due to chemical reaction between PH_3 and the red cells, damage to the vascular wall resulting in spastic and paralytic conditions and osmotic disturbance of permeability. The paralytic condition was also observed in our investigations.

Anorexia was noted in an accidental exposure of 14 men to PH_3 (PHILLIPS 1954). Anorexia was detected in our test animals also. According to MULLER (1940) congestion of all important organs are produced. Histopathological examination of the tissues showed in our experiments congestion in lungs, but not in other organs in the female albino rats used. WARITZ and BROWN 1975) found no histopathological effects in any of the tissues examined.

The LC_{50} and LC_{95} expressed as mg.hrs. per litre, the CT product, gives the actual gas concentrations and the time involved in the toxicity tests as the applied dosage will not persist as such due to sorption and leakage losses. Hence the ambient CT product as a parameter is more meaningful. The mean concentrations can also be computed from these data easily.

There was a variation in the LC_{50} values between the products A and B. This could be due to temperature effects. It is well known that toxicity increases with a rise in temperature. Product A was associated with a lower LC_{50} (0.22 mg.hr /L at 27°C) whereas product B was associated with a higher LC_{50} (0.36 mg. hr /L at 26.1°C). At the LC_{95} level, however, the difference was not so glaring (0.42 and 0.49 mg.hr /L for products A and B respectively. The LC_{50} of 0.44 μM/L/4 hr works out to a concentration of 10 ppm for male rats which reveals a lower tolerance to PH_3 (WARITZ and BROWN 1975).

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