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# Aluminum Phosphide Poisoning-Related Deaths in Tehran, Iran, 2006 to 2013

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Abstract: Metal phosphides such as aluminum phosphide are potent insecticides. This highly toxic substance is used for rice and other grains protection in Iran. Due to its high toxicity potential and easy availability, it is widely used as a suicide poison. This substance has no effective antidote and the incidence of deaths due to its poisoning is increasing day by day in Iran.

The present study was conducted to show the increasing incidence of fatal aluminum phosphide poisoning and its toxicological and forensic aspects in an 8-year study, 2006 to 2013. Autopsy sheets were reviewed and cases with the history of aluminum phosphide poisoning were selected. Toxicological analysis results, demographic and necroscopic examination findings were studied. A total of 51.8% of studied cases were female. Most of the cases were between 10 and 40 years old. The manner of death was self-poisoning in 85% of cases. Morphine, ethanol, and amitriptyline were the most common additional drugs detected in toxicological analysis. The incidence of fatal aluminum phosphide poisoning cases referred for phosphine analysis was 5.22 and 37.02 per million of population of Tehran in 2006 and 2013, respectively. The results of this study showed that in spite of ban and restrictions, there was a dramatic increase in the incidence of fatal aluminum phosphide poisoning in Tehran from 2006 to 2013. Safety alert should be highlighted in training program for all population groups about the toxic effects of aluminum phosphide tablets.

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Abbreviations: AIP = aluminum phosphide, DAD = diode array detector, FID = flame ionization detector, GC/MS = gas chromatography/mass spectrometry, HPLC = High performance liquid chromatography, HSGC = headspace gas chromatography, HS-GC/NPD = headspace-gas chromatography/nitrogen phosphorous detector, TLC = thin-layer chromatography.

## INTRODUCTION

luminum phosphide (AlP) and zinc phosphide are effective A fumigant pesticides and are used throughout the world to

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protect stored grains during storage and transportation from pests and rodents.<sup>1,2</sup> AlP exists as yellowish to dark gray granular or powdered solid which is formulated as tablets, pellets, or small sachets of powder.

Ammonium carbamate, ammonium bicarbonate, methanethiol (methylmercaptane), urea, and paraffin are added as additional materials to regulate phosphine gas release, moisture uptake, suppress flammability and to warn against phosphine presence in atmosphere.<sup>3-5</sup> It is well known that AlP is cheap and highly potent against many insect species and leaves little residue on rice and other food grains. Owing to these properties it is used extensively in developing countries as an effective grain fumigant.<sup>2</sup>

Phosphides are converted into toxic hydrogen phosphine (PH<sub>3</sub>) gas following contact with moisture, water, or hydrochloric acid in stomach content during their approved use or intentionally by suicidal ingestion. Pure phosphine gas is odorless and colorless. Technical grade phosphine has an odor similar to garlic or decaying fish due to impurities added in manufacturing processes.  $^{2,6-8}\!\!$ 

Phosphine is a respiratory poison. It inhibits mitochondrial oxidative phosphorylation by blocking cytochrome c oxidase, inhibits cellular respiration, and results in multi-organ damage and death.9 There is no specific antidote for phosphine gas poisoning and many patients do not survive despite intensive care.<sup>10,11</sup> AlP tablets, known as "rice tablets," are used to protect rice during long-term stock storage in Iran. Rice tablets are marketed in 2 forms in Iran; 3-gtablets containing AlP (Phostoxin, Celphos, Quickphos, and Phostek), which are highly toxic and their poisoning has a high mortality; herbal products which contain garlic and other herbal powders which are potentially harmless.<sup>12</sup> Phosphides do not show any evidence of danger to the population when they are used properly. Intentional and accidental poisoning with phosphides isdangerous.<sup>13</sup> In order to control and prevent acute deaths associated with the most toxic compounds, several countries use the legal regulation of pesticides trading and utilization.<sup>14</sup> Authorities banned AlP containing tablets in Iran since 2007.<sup>15</sup> Despite this regulation, continuing the use of this substance causes increase in the incidence of AIP poisoning and it is now one of the most common suicidal poisoning and cause of death in Iran.

Low survival rate following AIP poisoning and the magnitude of these poisoning-related deaths have drawn a great deal of attention in Iran, and this was the most important reason to design the present study in legal medicine organization, Tehran, Iran. Also few reports exist about forensic toxicology analysis and epidemiologic studies in AlP-related deaths<sup>7,10–12</sup>; however, the present study covers both issues in combination with each other. The purpose of the present study was to investigate phosphine poisoning-related deaths in Tehran, Iran in an 8-year interval. Toxicological analysis results, demographic and necroscopic studies' results, trend of fatal AIP poisoning and its prevalence study were included in our aim to perform the present study.

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# MATERIALS AND METHODS

This was a cross-sectional study of toxicological analysis results, demographic and necroscopic studies results, trend of fatal AIP poisoning and its prevalence of 2007 fatal phosphine poisoning cases admitted to Legal Medicine Organization, Tehran, Iran from December 30, 2005 to January 1, 2014. We retrospectively identified decedents with a principal diagnosis of AIP poisoning as cause of death. Detailed autopsy report had been archived for each case. These reports describe the autopsy procedure, give a list of medical diagnosis and a brief history of cases. Autopsy sheets were reviewed and cases with the history of AIP poisoning were selected consecutively to be investigated in the present study. Only cases with the history of AIP poisoning were included in the present study. Deaths due to other causes were excluded. Age, sex, toxicological analysis results, and necroscopic signals were variables of interest. Age range distribution of fatal phosphine poisoning cases referred for phosphine analysis was studied. To determine whether phosphine was listed as a contributing factor in death, forensic toxicology analyses were requested by supervised coroners. To address any sources of bias in determining the actual cause of death, systematic toxicological analyses were performed on liver, bile, stomach content, blood, and vitreous humor collected at autopsy examination in Forensic Toxicology Laboratory, Legal Medicine Organization, Tehran, Iran. Diagnosis of phosphine poisoning was based on the history given by relatives, autopsy examination, hospital reports, and finally toxicology analyses.

## **Ethical Statement**

According to the Legal Medicine Research Center Ethics Committee law, all personal information needed to perform any research studies on postmortem cases should be confidential. Information needed in the present study was based on previous autopsy and analytical toxicology reports, thus no ethical approval and patient consent are required.

# TOXICOLOGICAL ANALYSIS

# **Phosphine Analysis**

## Silver Nitrate and Ammonium Molybdate Tests

PH<sub>3</sub> gas was detected by silver nitrate (AgNO<sub>3</sub>) impregnated paper test qualitatively according to the analysis method described by Chugh et al.<sup>16</sup> This method is simple and sensitive for the detection of phosphine. For the detection of phosphine and phosphorus in stomach content, other biological and nonbiological samples ammonium molybdate test was used. About 50 g of homogenized liver tissue or mixed stomach content was put into a steam distillation flask. The sample was mixed with the same amount of water and acidified with dilute sulfuric acid. The mixture was distilled. The distillate was collected in an ice cold receiver. A piece of silver nitrate impregnated paper was put at the orifice of distillation flask. Black colored silver phosphate is the product of the reaction between silver nitrate and phosphine. Further confirmation of the presence of phosphine was done by converting the silver phosphate product to phosphomolybdate by dropping ammonium molybdate drops on silver phosphate. A blue color was formed by adding a drop of benzidine solution in the center of paper when exposed to ammonia fumes.2,16

# Headspace-Gas Chromatographic Analysis

Headspace-gas chromatographic technique using a nitrogen phosphorous detector (HS-GC/NPD) was used to confirm positive results according to the method described by Chan et al.<sup>17</sup> Liver and stomach content samples (which were available in  $-20^{\circ}$ C specimens archives) with positive results from silver nitrate and ammonium molybdate tests were analyzed using HS-GC/NPD technique. About 1 g of thawed liver and stomach content samples were mixed with 10% H<sub>2</sub>SO<sub>4</sub> for 40 sec in a completely sealed headspace vial. A headspace sampler combined with an Agilent GC7890A-NPD equipped with a HP-1 GC column (30 m length × 0.32 mm I.D. and film thickness 0.25 µm) was used under the following conditions: He carrier gas; injection volume 0.1 mL; oven temperature:  $60^{\circ}$ C for 1 min followed by  $10^{\circ}$ C per min up to  $150^{\circ}$ C with a 5 min hold; injector and detector temperatures were 150 and 250°C, respectively.

# **Other Drugs and Poisons Analysis**

## **Sample Preparation**

Liquid–liquid extraction was used to prepare biological samples (urine, blood, liver, bile, and stomach content) before toxicological analysis. Depending on the analyte chemical structure and analytical method used, different organic solvents such as chloroform, methanol, isopropanol, and diethyl ether were used in the cleanup and extraction of drugs from biological matrices. Vitreous humor and femoral vein blood (containing 1% W/V sodium fluoride) were used to detect ethanol and methanol using HSGC technique.

## Analysis

Thin-layer chromatography (TLC) technique was used as screening test for the qualitative analysis of drugs, poisons, and opioid alkaloids from biological matrices. Samples were analyzed with more sensitive and specific analytical methods. High performance liquid chromatography (Knauer, Germany) with a diode array detector (DAD) (Knauer DAD 2700, Germany) equipped with a quaternary pump (Knauer pump 1000, Germany) and a gas chromatography/mass spectrometry (GC/MS) (Agilent 7890A, USA) with a mass detector (5975C) were used as confirmatory methods for the confirmation of positive results obtained from the screening test. HSGC (Agilent 6890N, USA) equipped with flame ionization detector was used to analyze blood and vitreous humor samples for quantitative analysis of methanol and ethanol. Cecil 9000 spectrophotometer was used to detect carboxyhemoglobine in blood samples.

Biological samples were analyzed for the detection of heavy metals with Reinsch test. Reinsch test is an initial, simple, and qualitative method for the detection of heavy metals (eg, mercury, bismuth, arsenic, antimony, thallium) in biological and nonbiological samples (body fluids, homogenized tissue, food, and drink). The analysis is followed by confirmation technique using atomic absorption spectroscopy.<sup>18,19</sup>

Biological samples were analyzed for the qualitative analysis of cyanide using Prussian Blue test (a colorimetric method) and polarography/voltammetry (*Metrohm*797 analyzer) techniques.

Forensic autopsy reports and laboratory analysis sheets were reviewed from 2006 to 2013 to collect demographic data and other information needed to perform the present study. All data were analyzed using Chi-squared test SPSS<sup>18</sup> for the testing of significance in the trends of total referred cases for phosphine analysis, total number of positive phosphine tests in Tehran population, age range distribution, incidence of referred cases

for phosphine analysis, and positive phosphine tests in Tehran per 1 million of population from 2007 to 2013. *P* values of 0.05 were considered indicative of statistically significant difference.

#### RESULTS

#### **Toxicological Analysis Results**

#### Silver Nitrate, Ammonium Molybdate, and HS-GC/ NPD Tests Results

A total of 84.75% (1701) cases exhibited positive results for phosphine using silver nitrate and ammonium molybdate tests. Preliminary positive liver and stomach content samples for phosphine were analyzed to be confirmed by HS-GC/NPD method. Positive results were obtained for phosphine using this method too.

#### **Other Drugs and Poisons Analysis Findings**

Included in the present study were 385 (9.18%) cases with positive results for drugs and poisons other than phosphine. Table 1 shows the most common detected drugs and substances in postmortem samples of these cases. As it is shown in Table 1, morphine alone or in combination with other drugs was the most frequent drug detected in postmortem samples (180 cases, 8.97%), followed by ethanol (63 cases, 3.14%), amitriptyline (55 cases, 2.74), and other drugs and poisons. In many cases, more than 1 additional drug or substance were found in biological samples (Table 1).

#### **Case History Findings**

The manner of death was suicidal attempt in 85% of the study cases. Case history study of decedents is shown in Table 2.

#### Demographic and Epidemiologic Findings

In this study, there was a significant increase (P < 0.05) in the numbers of deaths due to phosphine poisoning in an 8-year study (Figure 1). Among 2007 cases, 1040 (51.82%) were female with female/male ratio 1.082. There was no significant difference between the number of males and females (P > 0.05). The mean age (standard deviation) of cases was 26.82 (10.58) years with the age range of 5 to 74 years. The majority of cases were young adults (Figure 2), and peak prevalence was seen in age groups of 10 to 40 years (1892 cases, ie, 94.27%). The incidence of referred cases and positive phosphine test cases showed an increasing trend (P < 0.05) from 2006 to 2013 per 1

**TABLE 1.** Additional Drugs and Substances Detected With Phosphine Gas in Biological Samples of Referred Cases to Toxicology Department, Tehran, Iran, 2006 to 2013

|                    | Year |      |      |      |      |      |      |      |       |
|--------------------|------|------|------|------|------|------|------|------|-------|
| Drug Name          | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | Total |
| Morphine           | 15   | 18   | 19   | 18   | 24   | 26   | 28   | 32   | 180   |
| Ethanol            | _    | 1    | 5    | 3    | 7    | 10   | 17   | 20   | 63    |
| Amitriptyline      | 2    | 1    | 3    | 6    | 6    | 9    | 13   | 15   | 55    |
| Tramadol           | _    | 3    | _    | 5    | 8    | 10   | 12   | 14   | 52    |
| Methadone          | _    | _    | _    | _    | 3    | 5    | 8    | 11   | 27    |
| Diazepam           | -    | -    | 2    | -    | -    | -1   | 2    | 5    |       |
| Strychnine         | _    | _    | _    | 1    | _    | _    | _    | _    | 1     |
| Cyanide            | _    | _    | _    | _    | _    | 1    | _    | _    | 1     |
| Chlorpyrifos ethyl | -    | -    | -    | -    | 1    | -    | -    | -    | 1     |

 TABLE 2. Case History Distribution of Aluminum Phosphide

 Poisoning-Related Deaths, Tehran, Iran, 2006 to 2013

| Case History         | Number (%)  |
|----------------------|-------------|
| Suicide attempt      | 1706 (85)   |
| Addiction            | 204 (10.16) |
| Psychiatric disorder | 42 (2.1)    |
| Alcoholism           | 38 (1.89)   |
| Unknown              | 17 (0.85)   |

million of Tehran population (Figure 3). No seasonal variation was observed for AlP poisoning (P > 0.05).

#### **Necroscopic Examination Findings**

Necroscopic signals obtained from observational and internal examination of cases are illustrated in Table 3.

#### DISCUSSION

The purpose of the present study was to investigate phosphine poisoning-related deaths in Tehran, Iran in an 8-year interval. Data obtained from the present study indicate that, in recent years, Iran has witnessed an increase in phosphine poisoning-related deaths. Poisoning as a consequence of suicidal and accidental ingestion of AIP is a frequent medico legal problem in Iran. Food and Drug Department of Iran restricted and announced a ban on the use and sell of metal phosphides,<sup>20</sup> but these substances are available abundantly and the mortality rate due to their poisoning is still high. Despite Asian countries, AIP is only available for qualified users in European countries such as UK, thus suicide by AIP ingestion is rare.<sup>21</sup>

Silver nitrate and ammonium molybdate tests were used to detect and confirm phosphine in biological samples (liver and stomach contents). Many studies had been done on AIP poison-ing in Iran and India.<sup>3,22–29</sup> Most of these studies reported and confirmed phosphine poisoning from clinical and paraclinical manifestations, relatives reports, statement from patients and death scene investigation. Systematic and chemical toxicological analysis of biological specimens in cases of AlP poisoning is underrepresented in previous studies.<sup>25,30,31</sup> Most of these studies focused on indirect phosphine poisoning detection by measurements of marker substances such as aluminum and phosphorus in biological samples; however, phosphine was detected in biological samples by HSGC in the study of Musshoff et al.<sup>5</sup> In the present study, phosphine was analyzed qualitatively in biological samples with other drugs and poisons in forensic toxicology laboratory. HS-GC/NPD technique is used as a confirmatory method for silver nitrate and ammonium molybdate tests in the present study.

Phosphine was detected in 51.82% of cases. There are many reasons for getting negative results in the remaining 48.18% of cases. Phosphine is a volatile substance; it can be evaporated from body during the period of death to autopsy, also in decontamination processes in hospital the concentration of phosphine declines in the body. The other reason for getting negative results is that there are other causes of death other than phosphine poisoning.

One of the variables that were studied in this paper was toxicological analysis for other drugs. Use of drugs by suicide

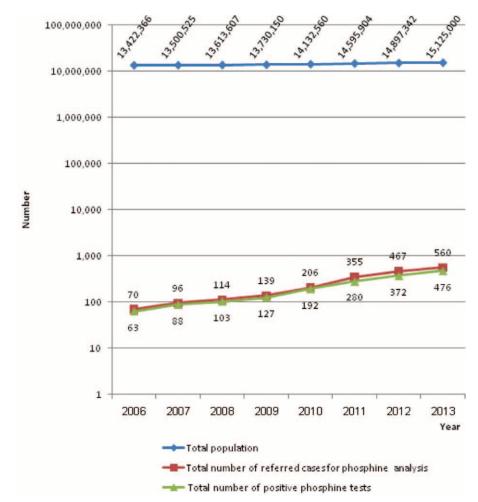


FIGURE 1. Trend of the total referred cases for phosphine analysis, total number of positive phosphine tests in Tehran population in an 8year study, Tehran, Iran, 2006 to 2013.

victims had been confirmed by previous studies.<sup>32</sup> Lahti and Vouri<sup>33</sup> stated that single and multiple toxicant poisonings can play significant role in accidental and suicidal deaths. The influence of alcohol and psychoactive drugs such as

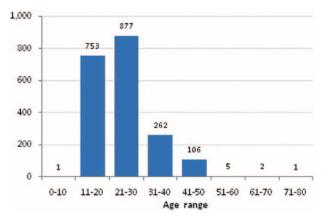
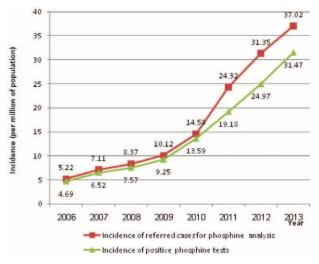


FIGURE 2. Age range distribution of fatal phosphine poisoning cases referred for phosphine analysis in Tehran, Iran in an 8-year study, Tehran, Iran, 2006 to 2013.

antidepressants, benzodiazepines and opioids in suicidal poisoning was discussed in a 10-year retrospective study.<sup>34</sup> Nowadays suicide methods had been changed and are different between countries. Acute opiate overdose is one of the most dramatic complications of drug abuse in Iran.<sup>35</sup> All of the suicidal cases in the present study showed positive results for at least 1 drug category.

Accidental and intentional exposure to AIP fumigants had been described by previous studies. A common method of suicide attempt in developing countries is pesticide poisoning.<sup>36</sup> Risk factors in suicidal cases may probably be underreported. There are many studies which described strong association between suicide mortality and a disease (mental or physical).<sup>37–40</sup> Suicide may be attempted by taking solid phosphides by mouth. Alp had been ingested as a poison for committing suicide in some countries such as Iran, India, and Morocco.<sup>3,22,23–29</sup> Fatal phosphine poisoning due to committing suicide accounted for 85% of total cases in this study. Clandestine production of methamphetamine can produce phosphine as a result of the reaction of iodine and red phosphorous in aqueous media. There are case reports of accidental inhalation of phosphine in these clandestinelaboratories.<sup>41,42</sup> Another way for accidental phosphine poisoning was reported among workers engaged in fuming of stored grains.<sup>43,44</sup> We do not



**FIGURE 3.** Trend of incidence of referred cases for phosphine analysis and positive phosphine tests in Tehran per 1 million of population in an 8-year study 2006 to 2013.

have any report or history of accidental poisoning in the present study, but we suppose that poisoning in lower age ranges (0-10 years) can be accidental.

According to the results of the present study, 2007 cases of phosphine poisoning-related deaths were referred for death investigation to Legal Medicine Organization, Tehran, Iran. Similar studies have reported AIP poisoning in other countries with less number of cases. One possible explanation for these differences can be governmental regulations for pesticides use. Hajouji-Idrissi et al<sup>45</sup> reported 28 cases of AlP poisoning admitted in a hospital in Morocco in a 10-year study. One hundred twenty cases of AIP poisoning were discussed by Jain et al<sup>46</sup> between 1993 and 2002 in the department of forensic medicine, Gandhi Medical College Bhopal. Murali et al47 reported 163 fatal phosphine poisoning in a 15-year experience. Also Bogle<sup>48</sup> reported 93 cases of AlP poisoning in a 4-year study. In agreement with our findings Murali et al stated that males did not outnumbered females, while male/female ratio was 2/1 in the study of Kapoor et al.<sup>22,47</sup> Maximum deaths belonged to the young age groups. Similarly the involvement of young age group with phosphide poisoning was recorded by other studies.<sup>22,47–49</sup> No significant difference in the number of deaths was seen in different seasons. This result was obtained by Murali et al too, but in some studies most of the AlP poisoning cases were recorded in the summer season.<sup>22,47</sup> Increasing

**TABLE 3.** Necroscopic Signals Found in Autopsy Examination of Fatal Phosphine Poisoning Cases, Tehran, Iran, 2006 to 2013

| Necroscopic Signal                                 | Number (%) |
|--|------------|
| Bluish discoloration of the skin                   | 15         |
| Bloody froth around the mouth                      | 20         |
| Garlic or decaying fish odor in stomach            | 50         |
| Congestion of gastric mucosa                       | 57         |
| Congestion in the trachea and lungs                | 98         |
| Moderate to severe congestion of livers and kidney | s 65       |

changes in the trend of phosphine-related deaths were observed in the present study. This increase shows that governmental restrictions for selling AIP tablets had not been effective so far and these tablets are cheap and easy available in Iran.

Pellets and sachets are produced for convenience and safety. Production of AlP sachets can lead to decrease severity of poisoning. During opening of these sachets for oral ingestion, AlP gets exposed to atmosphere moisture and phosphine gas escapes from the formulation thus its concentration decreases.<sup>50,51</sup> According to the study of Murali et al<sup>47</sup> change in the formulation of AlP from 3-g tablets to 10-g granulated powder in plastic sachets has led to a decline in the mortality rate of phosphide poisoning in India. Therefore, it can be one of the most important approaches in Iran's medical and pharmaceutical policy in the future to change AlP formulation.

The coroners' autopsy reports can make valuable information about the use of toxic substances for different purposes. Bluish discoloration of the skin, froth around the mouths, and garlic odor in AIP poisoning cases were reported by coroners in the present study. The results are in agreement with those of Jain et al.<sup>46</sup> Also Abder-Rahman<sup>52</sup> reported bloody exudate coming out of the mouth and nose of children poisoned with AlP. Some vital organs were found to be congested in the present study; this finding had also been observed in the previous studies.<sup>2,46,52,53</sup> Many studies pointed out to the organ toxicity of AlP poisoning. Heart and lungs are target organs for phosphine gas.<sup>54</sup> In experimental studies, phosphine can induce oxidative damage in liver, lungs, and brain of rats.<sup>2</sup> Histopathological examination of vital organs showed congestion, edema, and leukocyte infiltration especially in lungs, kidneys, and adrenal, which suggest cellular hypoxia in the study of Arora et al.<sup>49</sup> Anand et al<sup>55</sup> findings confirm the presence of congestion in liver and lungs of rats exposed to AIP. Previous studies suggest that a combination of energy insufficiency and oxidative stress could justify discussion about the pathophysiologic effects of phosphine toxicity.<sup>6,55</sup>

We should say that we encountered some limitations in the present study. Detailed and accurate history regarding the time and quantity of AIP tablets ingested, concomitant use of other drugs and resuscitative measures, decedents' hospitalization and survival time were not included in the death certificate of cases. Also to assess the mortality rate, we need to know the number of survivors in hospitals in Tehran. Larger studies in other provinces in Iran are required to assess this finding's generalizability, since efforts to reduce deaths from self-poisoning may benefit from concurrent efforts to reduce the import and production of AIP tablets.

In conclusion, data obtained from the present study showed that the trend of AIP fatality is increasing in Iran. This substance is not legally available for purchase by general public in Iran, although these tablets are imported to Iran illegally from other countries and are available in many herbal drug stores in tablet forms mostly packaging in aluminum cylindrical containers. Therefore, there is a growing need to substitute safer agents instead of metal phosphides and change the formulation available in Iran market. Also attention should be made to the fact that phosphides are highly lethal poisons with low safety and high mortality rates.

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