Chemical Reaction between Boric Acid and Phosphine Indicates Boric Acid as an Antidote for Aluminium Phosphide Poisoning

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Abstract: Objectives: Aluminium phosphide (AlP) is a fumigant pesticide which protects stored grains from insects and rodents. When it comes into contact with moisture, AlP releases phosphine (PH3), a highly toxic gas. No efficient antidote has been found for AlP poisoning so far and most people who are poisoned do not survive. Boric acid is a Lewis acid with an empty p orbital which accepts electrons. This study aimed to investigate the neutralisation of PH3 gas with boric acid. Methods: This study was carried out at the Baharlou Hospital, Tehran University of Medical Sciences, Tehran, Iran, between December 2013 and February 2014. The volume of released gas, rate of gas evolution and changes in pH were measured during reactions of AlP tablets with water, acidified saturated boric acid solution, acidified saturated boric acid solution, activated charcoal and acidified activated charcoal. Infrared spectroscopy was used to study the resulting probable adduct between PH3 and boric acid. Results: Activated charcoal significantly reduced the volume of released gas (P < 0.01). Although boric acid did not significantly reduce the volume of released gas, it significantly reduced the rate of gas evolution (P < 0.01). A gaseous adduct was formed in the reaction between pure AlP and boric acid. Conclusion: These findings indicate that boric acid may be an efficient and non-toxic antidote for PH3 poisoning.

Keywords: Antidotes; Aluminium Phosphide; Poisoning; Boric Acid; Phosphine; Activated Charcoal.

Advances in Knowledge
- The results of the present study show that phosphine (PH3) reacts with boric acid and produces a gaseous adduct.
- Activated charcoal was found to significantly reduce the volume of released PH3 gas, while boric acid significantly reduced the rate of gas evolution.
- The time taken for the production of a lethal volume of PH3 gas was 6.5–21 minutes.

Application to Patient Care
- The results of this study may be utilised by emergency medicine and poisoning centre staff to treat aluminium phosphide (AlP)-poisoned patients for the adsorption of released PH3 and to prevent further PH3 absorption. Treatment should comprise emergency oral administration of activated charcoal during a golden time period of no longer than 20 minutes post-AlP ingestion.
- The present study proposes boric acid as a new antidote for AlP poisoning; however, extensive in vivo studies are needed to confirm its effectiveness in animals and humans.

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Aluminium phosphide (AlP) is a fumigant pesticide often utilised to protect stored grains from insects and rodents. Although AlP is not toxic per se, the pesticide releases phosphine (PH₃)—a colourless, water insoluble, flammable and highly toxic gas—after coming into contact with water.¹⁻³ The gas is produced according to the following chemical equation:⁴

\[ \text{AlP} + 3\text{H₂O} \rightarrow \text{Al(OH)}₃ + \text{PH₃}↑ \]

While odourless in its pure form, PH₃ can smell of garlic or decaying fish due to the presence of impurities such as substituted phosphines and diposphines.¹² PH₃ is a Lewis base and a strong nucleophile. It is a reducing agent with a lone-pair electron which reduces cytochrome c oxidase and interferes with the electron transfer from complex III to complex IV of the mitochondrial respiratory chain, ultimately resulting in the inhibition of oxidative phosphorylation, adenosine triphosphate depletion and cell death.¹ AlP is a multiorgan poison which has toxic effects on the cardiovascular, respiratory, hepatic and gastrointestinal systems and induces acid-base disturbances.¹⁵⁻⁹ Myocardial damage is reported to be the primary cause of death in AlP poisoning.¹⁰¹¹ AlP poisoning is more prevalent in Iran and India.³³

Many reports have proposed experimental or individual case treatments for AlP poisoning, including digoxin; N-acetylcysteine; hyperbaric oxygen; magnesium (²⁴Mg²⁺)-carrying nanoparticles; intragastric irrigation with sweet almond oil; a combination of vitamin C and methylene blue; extensive gastric lavage with coconut oil and a sodium bicarbonate solution; 100 μL of concentrated 37% hydrochloric acid (Merck KGaA); activated charcoal with a 45–150 μm particle size and 850 m²g⁻¹ specific surface (ColorSorb® M5, Jacobi Carbons AB, Permatang Tinggi, Penang, Malaysia); AlP tablets (Phostoxin®, Alcan, Bucharest, Romania); pure AlP (MP Biomedicals LLC, Santa Ana, California, USA); and ammonium carbamate (Merck KGaA). A gas-collecting apparatus was assembled as follows: by a transparent flexible rubber tube, the side arm of an Erlenmeyer vacuum flask, placed on a magnetic stirrer, was connected to an upside-down water-filled graduated glass cylinder in a water basin. A combined pH meter electrode which simultaneously measured pH and temperature was tightly fitted to the mouth of the Erlenmeyer flask by means of a drilled gas-tight annular rubber stopper. The apparatus was placed under a ventilating laboratory hood to prevent the toxic gas from spreading.

Six experiments were performed as follows: a 1 g AlP tablet as an unbroken piece was added separately to 200 mL each of (1) distilled water (DW); (2) acidified DW; (3) 1% (weight [w]/volume [v]) activated charcoal in DW; (4) 1% (w/v) activated charcoal in acidified DW; (5) a saturated boric acid solution; and (6) an acidified saturated boric acid solution. For acidification of the solutions, 100 μL of concentrated 37% hydrochloric acid was added to the solutions to bring the pH to approximately 2.0, which is the approximate pH of the human stomach.¹ After the addition of an AlP tablet to each of the respective solutions, the gas-tight annular rubber stopper containing the combined pH meter electrode was immediately fitted tightly to the mouth of the Erlenmeyer flask and the mixtures were gently stirred by the magnetic stirrer. Each experiment was repeated five times. The volume of evolved gas and pH were recorded every minute and every 10 seconds, respectively, until the end of the reaction. The rate of gas evolution was determined by taking the first derivative of equations of respective released gas curves.

As each experiment was carried out at different times and with different ambient temperatures and pressures, all gas volumes were corrected for 310.15 Kelvin (K) or 37 °C and 101.325 kiloPascal (kPa) or
1 atmosphere; these values represent the normal temperature and the standard pressure, respectively, of and around the human body. This permitted a statistical comparison between the experimental groups. Correction of the evolved gas volume was done by using the following ideal gas law equation:

\[
\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}
\]

where \(P_1, V_1, T_1\) are the initial pressure in Pascal, volume in mL and temperature in K of the released gas, respectively, and \(P_2, V_2, T_2\) are 101.325 kPa, corrected volume in mL and temperature of 310.15 K, respectively. The ambient temperature and pressure of each experiment was measured using a multifunction digital altimeter (model KT808, DealeXtreme, Hong Kong, China). Because \(PH_i\) is a base, the pH of the reaction media was monitored continuously to evaluate the increase in pH of the mixtures.

Complementary experiments using the aforementioned apparatus were performed as follows: 0.56 g of pure AlP and 0.44 g of pure ammonium carbamate were separately added to 200 mL of DW, acidified DW, saturated boric acid and acidified saturated boric acid. The volume of released gas (if any) and pH were continuously recorded in these experiments. Each 1 g AlP tablet contained approximately 56% pure AlP and 44% ammonium carbamate by weight, which produces carbon dioxide (CO\(_2\)) and ammonia (NH\(_3\)) gases according to the following chemical equation:

\[
\text{NH}_2\text{COONH}_4 \rightarrow \text{CO}_2\uparrow + 2\text{NH}_3\uparrow
\]

Thus, one AlP tablet was expected to produce 236.3 mL of PH\(_3\), 137.8 mL of CO\(_2\) and 275.6 mL of NH\(_3\) and 650.0 mL of total gas at 25°C and 101.325 kPa. Due to the water solubility of PH\(_3\) (26 mL per 100 mL of water at 20°C and 101.325 kPa), CO\(_2\) (88 mL per 100 mL of water at 20°C and 101.325 kPa) and NH\(_3\) (34 mL per 100 mL of water at 20°C and 101.325 kPa), it was predicted that one AlP tablet would produce approximately 184.0 mL of PH\(_3\), 0.0 mL of CO\(_2\) and 208.0 mL of NH\(_3\) for 392.0 mL of total gas in 200 mL of DW at 20°C and 101.325 kPa if the reactions were complete.

Infrared spectroscopy was used to confirm the reaction between boric acid and PH\(_3\) gas and the formation of a phosphorous-boron bond. The infrared spectra of pure dry AlP, pure dry boric acid, a dry mixture of pure AlP and pure boric acid at a ratio of 1:1 (w/w) were obtained with an infrared spectroscope (FTIR-8400S, Shimadzu Corp., Kyoto, Japan) in a transmission mode between 500–4700 cm\(^{-1}\) with a resolution of 0.85 cm\(^{-1}\). All materials were used in powdered form. To study the effect of water on pure dry AlP, pure dry boric acid and a mixture of pure dry AlP and pure dry boric acid, the respective infrared spectra were obtained by spraying one puff (approximately 5 µL) of double DW on the respective dry samples.

Data were analysed using the Statistical Package for the Social Sciences (SPSS), Version 19 (IBM Corp., Chicago, Illinois, USA). The maximum volume \(V_{max}\) of released gas from each of the experiments was compared using a one-way analysis of variance with Scheffe’s post hoc test. Differences were regarded as significant at \(P <0.05\). Differences between rates of gas evolution in the experiments were determined by comparing the slopes of the respective rate curves using the Student t-test with the slopes of two lines considered as \(B_1\) and \(B_2\). The null hypothesis was that there would be no difference between these slopes \(B_1 = B_2\) and the alternative hypothesis was that there would be a difference \(B_1 \neq B_2\). In order to perform this analysis, a dummy variable (method) was first made and was coded one for line one and zero for line two. An additional variable (mettim, the product of method and time) was also made. Subsequently, method, time and mettim were used as predictors for slope comparisons. In the SPSS Syntax Editor Window (IBM Corp.), a programme was written and a statistical analysis was performed for two-by-two comparisons of the experimental groups.

## Results

The \(V_{max}\), maximum time needed to release \(V_{max}\), slopes of rate curves, difference between the initial and final temperatures of the reaction medium and the difference between the initial and final pH of the reaction medium of all experiments are summarised in Table 1. One AlP tablet produced 150.5 ± 2.2 mL of total gas and a maximum of 174.2 ± 1.5 mL of gas in 200 mL of DW and acidic DW, respectively, at 37°C and 101.325 kPa. The approximate time needed for the production of a lethal volume of gas was 6.5–21 minutes.

An AlP tablet in acidified DW produced significantly more gas than an AlP tablet in DW \((P <0.01)\). Moreover, the rate of gas evolution in acidified DW was significantly higher than in DW \((t = 11.76; P <0.01)\). The suspension of 1% (w/v) activated charcoal in DW significantly reduced the volume of released gas in comparison to the AlP tablet in DW \((P <0.01)\). However, the rate of gas evolution in a suspension of 1% (w/v) activated charcoal in DW was significantly higher than that of an AlP tablet in DW.
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A suspension of 1% (w/v) activated charcoal in acidified DW significantly reduced the volume of released gas in comparison to an AlP tablet in acidified DW ($t = 9.64; P < 0.01$). The rate of gas evolution in a suspension of 1% (w/v) activated charcoal in acidified DW was significantly higher than that of an AlP tablet in acidified DW ($t = 32.28; P < 0.01$). A suspension of 1% (w/v) activated charcoal in acidified DW significantly reduced the volume of released gas in comparison to an AlP tablet in acidified DW ($P < 0.01$). The rate of gas evolution in a suspension of 1% (w/v) activated charcoal in acidified DW was significantly higher than that of an AlP tablet in acidified DW ($t = 9.64; P < 0.01$).

Saturated boric acid solution did not significantly reduce the volume of released gas in comparison to an AlP tablet in DW ($P = 0.99$). However, the rate of gas evolution in a saturated boric acid solution was significantly slower than that of an AlP tablet in DW ($t = -11.50; P < 0.01$). The acidified saturated boric acid solution significantly reduced the volume of released gas in comparison to an AlP tablet in acidified DW ($P < 0.01$). The rate of gas evolution in the acidified saturated boric acid solution was also significantly slower than that of an AlP tablet in acidified DW ($t = -38.22; P < 0.01$). Gas evolution in the acidified saturated boric acid solution was significantly lower than that in a saturated boric acid solution ($P < 0.01$). The rate of gas evolution in the acidified saturated boric acid solution was significantly lower than in the saturated boric acid solution ($t = -19.74; P < 0.01$).

The infrared spectra of the pure dry and wet AlP, pure dry and wet boric acid and a dry and wet 1:1 mixture of pure AlP and pure boric acid were measured. For the pure dry AlP [Figure 1A], a weak peak was observed at 2,280–2,440 cm$^{-1}$, which was intensified after wetting the AlP [Figure 1B]. A comparison of pure dry [Figure 2A] and wet [Figure 2B] boric acid showed the production of no new peak in the latter spectrum. A comparison of the dry [Figure 3A] and wet [Figure 3B] mixture of pure AlP and boric acid indicated the production of three new peaks at 1,250 cm$^{-1}$, 1,350 cm$^{-1}$ and 1,440 cm$^{-1}$ in the latter wet mixture. An intensified peak at 2,280–2,440 cm$^{-1}$ was noted for the wet mixture in comparison to the dry mixture.

Table 1: $V_{max}$, $t_{max}$, slopes of rate curves, temperature differences and pH differences of all experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mean $V_{max}$ in mL ± SD</th>
<th>$t_{max}$ in minutes</th>
<th>Slope of rate curve in mL min$^{-1}$</th>
<th>Mean $\Delta t$ in °C ± SD</th>
<th>Mean $\Delta p$H ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. AlP tablet with DW</td>
<td>150.5 ± 2.2</td>
<td>64.5</td>
<td>-0.0736</td>
<td>1.8 ± 0.0</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>2. AlP tablet with acidified DW</td>
<td>174.2 ± 1.5*</td>
<td>67.4</td>
<td>-0.0786§</td>
<td>3.6 ± 1.2</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>3. AlP tablet with 1% activated charcoal in DW</td>
<td>114.4 ± 2.0*</td>
<td>52.2</td>
<td>-0.0939§</td>
<td>5.0 ± 1.6</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>4. AlP tablet with 1% activated charcoal in acidified DW</td>
<td>129.8 ± 0.5†</td>
<td>56.3</td>
<td>-0.0838¶</td>
<td>4.8 ± 0.4</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>5. AlP tablet with saturated boric acid</td>
<td>149.0 ± 5.6</td>
<td>67.2</td>
<td>-0.0683§</td>
<td>6.7 ± 1.3</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>6. AlP tablet with acidified saturated boric acid</td>
<td>136.3 ± 2.4†</td>
<td>71.2</td>
<td>-0.0558¶</td>
<td>6.8 ± 0.2</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>7. Pure AlP with DW</td>
<td>ND</td>
<td>NM</td>
<td>NC</td>
<td>0.9 ± 0.1</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>8. Pure AlP with acidified DW</td>
<td>ND</td>
<td>NM</td>
<td>NC</td>
<td>0.8 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>9. Pure AlP with saturated boric acid</td>
<td>ND</td>
<td>NM</td>
<td>NC</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>10. Pure AlP with acidified saturated boric acid</td>
<td>ND</td>
<td>NM</td>
<td>NC</td>
<td>1.7 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>11. Ammonium carbamate in DW</td>
<td>ND</td>
<td>NM</td>
<td>NC</td>
<td>1.2 ± 0.1</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>12. Ammonium carbamate in acidified DW</td>
<td>ND</td>
<td>NM</td>
<td>NC</td>
<td>1.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>13. Ammonium carbamate in saturated boric acid</td>
<td>ND</td>
<td>NM</td>
<td>NC</td>
<td>0.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>14. Ammonium carbamate in acidified saturated boric acid</td>
<td>ND</td>
<td>NM</td>
<td>NC</td>
<td>0.7 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
</tbody>
</table>

$V_{max}$ = maximum of released gas in each experiment; $t_{max}$ = maximum time needed to release $V_{max}$; $\Delta t$ = difference between initial and final temperatures of reaction medium in each experiment; $\Delta p$H = difference between initial and final pH of reaction medium in each experiment; SD = standard deviation; AlP = aluminium phosphide; DW = distilled water; ND = not detected; NM = could not be measured; NC = not calculable.

*Significantly different from experiment 1 ($P < 0.01$). †Significantly different from experiment 2 ($P < 0.01$). ‡Significantly different from experiment 5 ($P < 0.01$). §Significantly different from experiment 1 ($P < 0.01$). ¶Significantly different from experiment 2 ($P < 0.01$). \Gas evolution was too rapid and small to be detected.

The Discussion section of the paper would follow this point.
to cytochrome \( c \) oxidase \((E_0 = +0.29 \text{ V})\) and interferes with electron transfer in the mitochondrial respiratory chain.\textsuperscript{10} Theoretically, an electron acceptor stronger than cytochrome \( c \) oxidase can protect cytochrome \( c \) oxidase against \( \text{PH}_3 \) which might prevent or reduce the inhibition of cellular respiration.\textsuperscript{10} With an empty \( p \) orbital, boric acid seems to adequately fulfill this theory.\textsuperscript{1} The possibility of a reaction between \( \text{PH}_3 \) and boric acid forming an adduct of \( \text{H}_3\text{P-B(OH)}_3 \) has been recently proposed; in this theoretical reaction, \( \text{PH}_3 \), as a nucleophile and Lewis base, neutralises boric acid, as an electrophile and Lewis acid, and a \( \text{H}_3\text{P-B(OH)}_3 \) adduct is formed.\textsuperscript{1}

One AlP tablet was predicted to produce approximately 392.0 mL of total gas in 200 mL of DW at 20 °C and 101.325 kPa.\textsuperscript{4} However, in the present study, one AlP tablet produced less total gas in 200 mL of DW and acidic DW, respectively, at 37 °C and 101.325 kPa. This may be due to the incompleteness of this reaction at these conditions. The results also showed that activated charcoal significantly reduced the volume of released gas from AlP tablets. Previous studies have shown that activated charcoal is a universal antidote which adsorbs many poisons as well as some gases; as such, it is often used in poisoning emergency centres for gastrointestinal decontamination.\textsuperscript{23–25} Although it was expected that more gas would evolve in the saturated boric acid solution as the solubilities of \( \text{CO}_2 \) and \( \text{NH}_3 \) in this solution are less than those in DW, this did not occur. It seems that boric acid reacts with these gases and traps them in solution.\textsuperscript{1,26} Another explanation may be the production of less \( \text{CO}_2 \) and \( \text{NH}_3 \) in this solution due to reduced water molecules around the AlP tablet.\textsuperscript{1,26}
In general, $\text{PH}_3$ gas has two infrared peaks at between 950–1,250 cm$^{-1}$ and 2,280–2,440 cm$^{-1}$ which are related to the bending and stretching of the phosphorus-hydrogen bond.21 In the current study, pure wet AlP showed a more intensified peak at 2,280–2,440 cm$^{-1}$ than dry pure AlP. This may be due to the production of more $\text{PH}_3$ gas after wetting pure dry AlP. The peak at 950–1,250 cm$^{-1}$ related to the phosphorus-hydrogen bond bending of $\text{PH}_3$ also seemed to be overlapped by the AlP peak. For the 1:1 (w/w) mixture of pure AlP and pure boric acid, an intensified peak at 2,280–2,440 cm$^{-1}$ was noted for the wet mixture in comparison to the dry mixture; this seems to be due to an overlap of a boric acid peak in this region with that of $\text{PH}_3$. The production of new peaks after wetting the dry mixture strongly suggests the formation of a new chemical product. Along with this infrared spectroscopic data, the gentler slope of the gas evolution rate curve in the boric acid solution suggests the formation of a gaseous adduct during the reaction between $\text{PH}_3$ and boric acid, which is comparable to the reaction of $\text{PH}_3$ and $\text{BCl}_3$ and subsequent production of a $\text{H}_2\text{P-BCl}_3$ adduct.22 The reaction product of AlP ($\text{PH}_3$) and boric acid in the present study had very similar infrared spectroscopic absorption peaks (in the region of 1,250–1,450 cm$^{-1}$) to the reaction product of $\text{PH}_3$ and $\text{BCl}_3$, indicating the formation of a phosphorous-boron bond.21

In addition, the authors of the current study found that breaking the AlP tablet into fragments reduced $\text{PH}_3$ gas evolution in comparison to an equiweight unfragmented AlP tablet, with more fragments producing less $\text{PH}_3$ gas. However, the powdered form of the AlP tablet produced too little gas which the gas-collecting assembly was unable to collect and hence the data were not presented. This greatly reduced production of gas may be due to the surface chemistry of AlP tablets and may also explain why reduced mortality and fewer systemic effects have been reported among individuals who have ingested fragmented or powdered forms of AlP.27,28 In nearly all animal studies, the AlP tablet is administered by gastric gavage in powdered form in a carrier such as peanut oil, almond oil or normal saline.15,16,29 This method of poisoning may therefore be incorrect because fragmentation or powdering interferes with $\text{PH}_3$ gas evolution. Thus, examining oral AlP poisoning in animal studies is very difficult; instead, it is recommended that the animals be poisoned by $\text{PH}_3$ gas. In addition, AlP tablets contain ammonium carbamate which produces $\text{NH}_3$ and CO$_2$ gasses when in contact with water; these gasses also interfere with the results of AlP poisoning studies and should subsequently be excluded.

It has been previously shown that ingestion of AlP in as low a dose as 150–500 mg is lethal to human beings.2 These amounts are equivalent to 49.3–164.2 mg of $\text{PH}_3$ gas. Therefore, 150–500 mg of AlP was deemed approximately equivalent to 26–87 mL of gas in the acidic environment of the stomach, at 37 °C and under 101.325 kPa. According to the current study, the approximate time needed for the production of a lethal volume of $\text{PH}_3$ gas was 6.5–21 minutes. As such, the optimal or ‘golden’ time period to save a poisoned human seems to be up to 20 minutes post-ingestion of 150–500 mg of AlP, which is a very short time frame to ensure that the patient receives an antidote. After this, a lethal amount of $\text{PH}_3$ is released and absorbed and it is unlikely that any therapy will be effective. Treatment should therefore comprise emergency oral administration of activated charcoal during this ‘golden’ time period. The present study indicates that boric acid may be a new antidote for AlP poisoning; however, extensive in vivo studies are needed to confirm its effectiveness in animals and humans. Overall, the current study showed that although saturated boric acid solution did not significantly reduce the volume of released gas in comparison to DW, acidified saturated boric acid solution significantly reduced the volume of released gas in comparison to acidified DW. These results, along with a higher rate of gas evolution in the former solution and infrared spectroscopic data, indicate the formation of a gaseous product with a stronger hydrogen bond in acidic boric acid than in boric acid. This suggests that this gaseous product has a high vapour pressure. A limitation of the current study was that neither the volume of $\text{PH}_3$ consumed nor the amount of product produced was measured; these should be taken into account during future research.

**Conclusion**

The results of this study indicate that $\text{PH}_3$ reacts with boric acid and produces a gaseous adduct. The approximate time needed for the production of a lethal volume of $\text{PH}_3$ gas was 6.5–21 minutes. Activated charcoal significantly reduced the volume of released gas. These findings suggest that AlP-poisoned individuals should be treated with emergency oral administration of activated charcoal during a ‘golden’ time period of up to 20 minutes post-ingestion for adsorption of released $\text{PH}_3$, and the prevention of further $\text{PH}_3$ absorption. The present study indicates that boric acid may be a new antidote for AlP poisoning, although further research is needed to confirm its effectiveness.
CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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References


