A FIELD TEST FOR THE DETECTION OF PHENYL MERCURY ACETATE (PMA) IN AIR AND SURFACE RESIDUES

SUMMARY

A rapid and simple method for the detection of an organo mercury pesticide phenyl mercury acetate (PMA) is described. The method is based on the ligand-exchange reaction e.g., hexacyanoferrate (III) \( \text{K}_3\text{Fe(CN)}_6 \) to exchange cyanide ions with chromogenic organic ligand, succinyl dihydroxamic acid (SDHA) in presence of mercury. In the reaction the colourless SDHA reacts with yellow \( \text{K}_3\text{Fe(CN)}_6 \) to give a greenish blue coloured complex in a slightly acidic solution containing PMA. The reaction has also been effectively applied to detect other mercury containing compounds in air, soil and folicies. In air, at a velocity of \( \sim 0.2 \) lit/min of the impinging air at a reaction temperature of \( \sim 70^\circ\text{C} \), PMA as low as 0.015 \( \mu \)g could easily be detected after 3 minutes exposure. The limit of identification and limit of dilution were found to be 0.33 \( \mu \)g (PMA) and 1 : 150,000 respectively.
A FIELD TEST FOR THE DETECTION OF PHENYL MERCURY ACETATE (PMA) IN AIR AND SURFACE RESIDUES

Phenyl mercury acetate (PMA), an organomercury pesticide also known as 'Tag HL 331' is a powerful eradicant fungicide as well as a selective herbicide used in lawns for the control of crabgrass. The use of organomercury compounds as pesticides dates from 1914 when Riehm, E. reported that chlorophenol mercury was effective against bunt of wheat. The first use of phenyl mercury acetate as herbicide was by De France, J.A., against crabgrass. It is sold in India under the trade name of Parasan-one or PMA 1% D.S. to use as seed, turf, foliage, and industrial fungicide. It is an effective seed dresser to protect cereals from fungus diseases such as smut and bunt; and is effective in combating attack by fungal spores. In soil all the organomercury compounds are decomposed to mercury salts or to metallic mercury which are the active fungicides (1-7). PMA is also used to protect oil based paints (3).

The wide usage of mercury compounds in agriculture is a major aspect of environmental pollution by mercury. Several outbreaks of poisoning have been described as a consequence of such use of mercury compounds. Several hundred persons have become poisoned and many have died in Guatemala, Iraq and Pakistan following the consumption
of treated seeds (8-10). Poisoning by means of mercury compounds have been known since the time of the ancients. However, these compounds have received more recent acclaim as toxic compounds in connection with their existence as pollutants in various rivers and streams. Mercury compounds are reported to be retained by fish and eventually transmitted to humans. A recent report indicated that very low mercury concentrations (of the order of 0.1 μg/litre, often found in coastal waters) could inhibit photosynthesis and transpiration in phytoplankton (11-14).

Organomercury compounds are much more toxic to man and animals than inorganic mercury (12). These compounds continue to concentrate in the human body and eventually destroy the brain and central nervous system (3). The most important routes of its entry into living system are via the lungs and absorption through the skin (15-19). After absorption, it circulates in the blood and stored in the liver, kidney, spleen and bone. Chronic mercury poisoning includes the symptoms of loss of appetite, salivation, gingivitis, nutritional disturbances, renal damage, anaemia, stomatitis, chronic nasal catarrh, ocular lesions, constriction of the visual field, difficulty in speaking and nervous system damage (15-18,20,21). Mutagenic effect of PMA in different organism is also reported (21,22). A disease called "Minimata disease" has been reported in literature which is associated with
the ingestion of fish collected from mercury polluted waters. The main symptom of the disease is a 'CNS' disorder. A cerebral palsy-like disease referred to as "congenital Minimata disease" which includes paresthesias, loss of vision and hearing, incoordination and intellectual deterioration (23).

Acute mercury intoxication is characterized by pharyngitis, dysphagia, abdominal pain, nausea and vomiting, bloody diarrhea and shock, but the most characteristic effect is the so-called 'sublimate nephrosis' which depending on its severity, results in oliguria, anuria or death (16). The threshold limit value for organomercury compound is $10 \mu g/m^3$ by absorption through the skin (18).

The importance of PMA as a health hazard has led to the development of various methods for the detection of PMA in low concentrations; of these most widely used are colorimetric methods (24-27). Various other methods including gas chromatography (28), flow injection analysis (29), polarography (30), atomic absorption spectrophotometry (31), x-ray fluorescence spectrophotometry (32), neutron activation analysis (33), potentiometry (34), high performance liquid chromatography (35), ultra violet spectrophotometry (35), photoacoustic spectrometry (36), amperometry (37), combustion methods (38) and visual spectrophotometry (24,39-43) have been reported for the detection and determination of PMA and mercury compounds. Few methods have been reported which involve ligand exchange
reactions, e.g., hexacyanoferrate (II) \([\text{K}_{4}\text{Fe(CN)}_6]\) is known to exchange cyanide ions with chromogenic organic ligands such as p-nitrosophenyl amine (41), isonicotinyl hydrazide (42) and \(\alpha,\alpha'\) dipyridyl (43) to produce coloured complexes in presence of mercury (II) ion.

Due to autocatalytic reaction of mercury it takes 24-30 hours for the complete colour development, therefore a reliable quantitative determination of PMA could not be achieved in a short time. Though the above reagents (41-43) have been used for the determination of mercury, they suffer from various critical parameters such as time, temperature, quenching process, etc. Hence in the present investigation this reaction could not be used for the quantitative determination of PMA, but the method can easily be applied for the detection of PMA in air and surface residues.

In the present investigation hexacyanoferrate(III) \([\text{K}_3\text{Fe(CN)}_6]\) and a new chromogenic organic ligand succinyl dihydroxamic acid (SDHA) have been used for the detection of PMA. The method is based on the ligand-exchange reaction, e.g., hexacyanoferrate (III) \([\text{K}_3\text{Fe(CN)}_6]\) to exchange cyanide ions with chromogenic organic ligand SDHA in presence of PMA. In this reaction the colourless SDHA reacts with yellow \(\text{K}_3\text{Fe(CN)}_6\) to give a greenish blue coloured complex in a slightly acidic solution containing PMA. In air, as low as 0.015 \(\mu\)g of PMA could be detected at a velocity of \(\sim 0.2\) lit/min of
the impinging air after 3 minutes exposure at a reaction temperature of \( \sim 70^\circ C \). The limit of identification and dilution limit were found to be 0.33 \( \gamma \) PMA and 1:150,000 respectively.

**EXPERIMENTAL**

**Apparatus:**

Midget impingers of 35 ml capacity, a vacuum pump, spot plates and micro test tubes were used for the detection of PMA in air and surface residues.

**Reagents:**

Standard phenyl mercury acetate (PMA) solution: A 1 mg/ml solution of PMA was prepared in ethanol. Appropriate dilution of the stock was made for the preparation of working standard solutions.

Hexacyanoferrate (III) \( [K_3Fe(CN)_6] \) solution: A 1% solution of \( K_3Fe(CN)_6 \) was prepared in demineralized water.

Succinyl dihydroxamic acid (SDHA) (44):

**Preparation:**

The reagent was prepared by dropwise addition of 17.2 gms of diethyl succinate to the ammoniacal solution of 13 gms of hydroxyl amine hydrochloride with vigorous stirring at 5°C. The white precipitate of succinyl dihydroxamic acid obtained was filtered and crystallized twice with demineralized water (m.p. 164-166°C). The compound is stable for long periods.
Formula of SDHA:

\[
\begin{align*}
&CH_2 - C - N - H \\
&\text{0} & \text{OH}
\end{align*}
\]

A 0.1% aqueous solution of SDHA was used as a reagent.

PMA 1% D.S. (President Industries, Ahmadabad, India) - A technical grade fungicide.

Parasan-one (Hyderabad Chemical Supplies, Pvt. Ltd., India): A technical grade fungicide.

Parad tablets (Zandu Pharmaceutical works, Ltd., India): Mercury containing tablets used for controlling pests in stored grains.

All reagents unless mentioned otherwise used were of AnalaR grade.

Procedure:

A. Detection of PMA in air:

An impinger containing a known aliquot of PMA was connected to another impinger with the reagent solution containing equal volumes of 1% $K_3Fe(CN)_6$ and 0.1% SDHA. These two impingers were then connected to a source of suction. When lower PMA concentrations were
dealt with, heating accelerated the reaction, hence the
leapiner containing reagent solution was kept in a water
bath (\( \sim 70^\circ C \)). The PMA solution was heated by gradually
raising the temperature and air was drawn through
leapiners at a rate of \( \sim 0.2 \) lit/min for 3 minutes. The
change in yellow coloured reagent solution into greenish
blue colour indicated the presence of PMA.

8. **Spot test for the detection of PMA:**

A drop of test solution of PMA was placed on a
spot plate or in a micro test tube, then a drop of
\( \text{K}_4\text{Fe(CN)}_6 \), followed by one drop of SDMA was added. The
mixture was warmed in an oven at \( \sim 70^\circ C \) for \( \sim 10 \) minutes.
Depending on the quantity of PMA from which mercury was released, a greenish blue colour was obtained, which
indicated the presence of PMA. For very small amounts
of PMA a blank test was preferred.

**Application of the method:**

**Detection of mercury vapours liberated from Parad:**

Parad tablets are widely used in homes for
protecting grains from insects. At room temperature
these tablets liberate sufficient amount of mercury
vapours to kill insects but it could be harmful to human
beings when inhaled constantly. Hence the proposed method
has been used to detect mercury vapour in air.
The contaminated air sample from a grain container in which parad tablets were used for controlling insects, was drawn into an impinger kept on a water bath (70°C) containing test solutions as described in procedure for the detection of PMA in air. The colour of the test solution turned from yellow to greenish blue, indicating the presence of mercury in the air sample.

**Detection of PMA in soil and foliages:**

The soil samples from lawn and foliage samples from field over which PMA 1% D.S. or Parasan-one was sprayed were collected. A 0.2 g of soil sample or 0.5 g of foliage sample was thoroughly washed and filtered with ethanol. The filtrate was then used for the detection of PMA following the described procedure B. The formation of greenish blue colour indicated the presence of PMA in the samples. A blank test was also carried out throughout the procedure.

**RESULTS AND DISCUSSION**

The proposed method is based on the ligand-exchange reaction. The colourless SDHA solution reacts with yellow $\text{K}_3\text{Fe(CN)}_6$ in presence of PMA, giving a greenish blue product. Greenish blue colour was not observed when PMA was added independently to $\text{K}_3\text{Fe(CN)}_6$ or SDHA solution, proving that the colour formation is due to the ligand-exchange reaction between $\text{K}_3\text{Fe(CN)}_6$ and SDHA in the presence of mercury in PMA.
The reaction was also carried out with hexacyanoferrate (II) \( K_4Fe(\text{CN})_6 \) and sodium nitroferricyanide dihydrate, but the intensity of the coloured product was found to be less than hexacyanoferrate (III). Hence, the present experiment was carried out with hexacyanoferrate (III) \( K_3Fe(\text{CN})_6 \). The SDHA which has been earlier used in this laboratory as a reducing agent for the determination of organophosphorus pesticides (44) is now successfully used as a chromogenic organic ligand for the detection of PMA.

**Reaction Mechanism:**

The reaction of hexacyanoferrate (II) with an organic ligand in presence of mercury is reported to take place in the following manner (27,41-43).

\[
\text{Fe(\text{CN})}_6^{4-} + H_2O \rightleftharpoons [\text{Fe(\text{CN})}_5\cdot H_2O]^{3-} + \text{CN}^- \quad \text{(1)}
\]

(Slow)

\[
[\text{Fe(\text{CN})}_5\cdot H_2O]^{3-} + L \rightarrow [\text{Fe(\text{CN})}_5\cdot L]^{3-} + H_2O \quad \text{(2)}
\]

(Organic ligand)

\[
\text{CN}^- + H_2O \rightarrow HCN + OH^- \quad \text{.. .. .. .. .. \ (3)}
\]

Mercury catalyses the decomposition of hexacyanoferrate (II) in the following manner:

\[
\text{Hg}^{2+} + \text{Fe(\text{CN})}_6^{4-} + H_2O \rightarrow [\text{Fe(\text{CN})}_5\cdot H_2O]^{3-} + \text{HgCN}^+ \quad \text{.. \ (4)}
\]

\[
\text{HgCN}^+ + H^+ \rightarrow \text{Hg}^{2+} + \text{HCN} \quad \text{.. \ .. \ (5)}
\]
The aquopentacyanoferrate (II) reacts with organic ligands to produce coloured products. In the proposed method it is likely that similar reaction takes place using hexacyanoferrate (III) and an organic ligand succinyl dihydroxamic acid (SDHA) in presence of mercury liberated from PMA to give greenish blue colour for the detection of PMA.

**Effect of pH:**

It was observed that the maximum colour of the sample was obtained in the pH range of 3-6. The colour decreased at lower pH, whereas at higher pH no colour was obtained. However, it was found that the reagents used as test solution themselves gave pH ~ 5.5, which was sufficient for the colour development.

**Effect of temperature and time:**

The effect of temperature and time for the reaction is reported to be critical (41-43). It was observed that at thermostated temperature (20 - 50°C), it took 40 - 60 minutes for the colour development, whereas at 70 - 90°C it took only 2-3 minutes for colour development. Longer heating time caused decomposition of the greenish blue complex and an increased blank was also observed, hence the experiment was carried out in a water bath or oven at 70°C for 2-10 minutes in case of detection in air or on spot plate.
The comparison of the proposed method with the reported colorimetric spot tests (24,27) shows a comparable sensitivity (Table I). It was found that other pesticides and ions such as organochlorine pesticides, organophosphorus pesticides, carbamates, chlorides, nitrate, phosphate, etc. do not interfere with this reaction. Ag and Pd (II) gives analogous reaction. Fe$^{2+}$ and Fe$^{3+}$ ions interfere due to the formation of coloured precipitate. The greenish blue coloured complex formed in presence of PMA was stable for more than a week.

CONCLUSION

The proposed test for the detection of PMA in air and surface residue is rapid, simple and reproducible. It can be successfully used to detect PMA technical grade fungicide formulations. This method can also be used for the detection of other organomercury compounds such as phenyl mercury borate, phenyl mercury nitrate, etc.
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test for</th>
<th>Details of method</th>
<th>Result</th>
<th>I.L.a</th>
<th>D.L.b</th>
<th>Principal interferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphthylcarbazone and organomercurials</td>
<td>Hg(I) or Hg(II) 0.2 M HNO₃ on paper</td>
<td></td>
<td>Blue to violet spot</td>
<td>1.00</td>
<td>5x10⁻⁴</td>
<td>Cr(VI), Mo(VI), Au; much V(V) and Fe(II). Interference by Cr(VI) can be eliminated by reduction with DO₃, and that of Mo(VI) by complexing with oxalate.</td>
</tr>
<tr>
<td>Dithizone</td>
<td>-do-</td>
<td>0.5 M HNO₃; Hg concn. must be very low; extract intoCCI₄ phase</td>
<td>Yellow Hg(I) orange Hg(II) colour in CCl₄ phase</td>
<td>0.25</td>
<td>1.1x10⁻⁵</td>
<td>Several metals, particularly Cu, Ag, Au, Bi, Pd.</td>
</tr>
<tr>
<td>p-Dimethylamino-     benzylidene- rhodanine</td>
<td>Hg(II)</td>
<td>Weakly acidic (pH&lt;7); add 3 drops saturated. NaAc if Cl⁻ is present; on spot plate.</td>
<td>Reddish violet suspension or ppt.</td>
<td>0.33</td>
<td>1.5x10⁻⁵</td>
<td>Ag in particular; also Au, Cu(I), Pt, Pd; Cu(II) interference only in high concn. Ag can be removed by pptn. with HCl.</td>
</tr>
<tr>
<td>Tin(II)chloride + Hg(II) or aniline</td>
<td>-do-</td>
<td>Acid solution; on paper</td>
<td>Grey to black spot</td>
<td>1.00</td>
<td>5x10⁻⁴</td>
<td>Cu, Au, Mo; much Ag, Fe, Pt, Pd.</td>
</tr>
<tr>
<td>Ferrocyanide ion + κ₂-dipyridyl organic materials</td>
<td>Hg(II) in</td>
<td>Heat 1 drop each of K₄Fe(CN)₆, NH₃ and κ₂-dipyridyl in micro test tube; Fe⁺⁺⁺ liberated.</td>
<td>Pink to red colour at once or within few minutes</td>
<td>2.00</td>
<td>2.5x10⁻⁴</td>
<td>Ag and Pd(II) give analogous reaction</td>
</tr>
<tr>
<td>Ferricyanide ion + SDMA (Present method)</td>
<td>Hg(II) and PMA</td>
<td>Heat 1 drop each of pot. ferricyanide and SDMA in micro test tube or on a spot plate.</td>
<td>Green to blue colour at once or within a few minutes</td>
<td>0.20</td>
<td>2x10⁻⁵</td>
<td>Ag and Pd(II) give analogous reaction. Commonly used pesticides do not interfere.</td>
</tr>
</tbody>
</table>

* - Identification limit in ′y;  ** - Dilution limit.
REFERENCES


