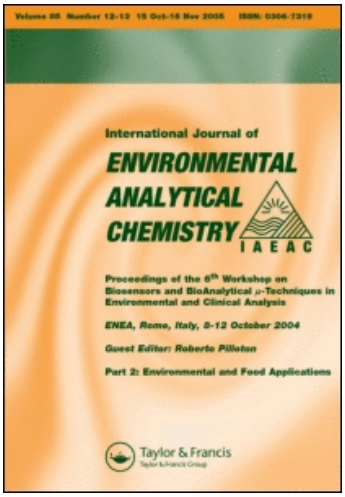


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# Various Means for the Detection of Organophosphorous Compounds

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# Various Means for the Detection of Organophosphorous Compounds

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Several reaction principles can be used for the detection of organophosphorous compounds. E.g. the "molybdenum blue" method, the colorimetric and fluorimetric variants of the Schoenemann reaction, electrochemical detection of cyanides upon reaction with certain oximes, photoemission of exitated HPO molecules in a hydrogen rich flame and the well known enzymatic detection of cholinesterase inhibiting compounds.

Various detection systems have been developed varying from simple, manually operated devices to more sophisticated and continuous functioning detectors.

These well established detection principles and some new emerging techniques, like microsensors using the surface acoustic wave technique, will be discussed.

## 1. INTRODUCTION

The increasing use of toxic (thio)phosphonates, phosphates and carbamates as pesticides, insecticides, corrosion inhibitors and anti-knock compounds, as well as the potential use of organophosphorous compounds as nerve agents have necessitated the development of highly sensitive and fast reacting detector devices. Before World War II the presence of chemical warfare agents was to be detected with

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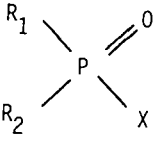
biological indicators (e.g. birds were taken to the battlefront) or by their olfactory properties. The days have passed that these methods can be employed without causing any harm. In the Netherlands, especially at the Prins Maurits Laboratory TNO, various systems have been developed for the detection of chemical warfare agents, that are of paramount importance as an integral part of a total defence system against chemical warfare. In this paper some past, present and future principles of detection will be reviewed.[1,2]

The organophosphorous compounds considered can be described with the general formula:




in which $R_1$ and $R_2$ are alkyl, alkoxy or iminogroups and X is a leaving group. Well-known examples of nerve agents are tabun, sarin and soman (G-agents) and VX (V-agent). Their structure is given in Table I.

**Table I** The structure of some nerve agents

| Nerve agent | $R_1$ | $R_2$ | X |
|---|---|---|---|
| tabun | $N(Me)_2$ | Et | CN |
| sarin | $OCH(Me)_2$ | Me | F |
| soman | $OCH(Me)C(Me)_3$ | Me | F |
| VX | OEt | Me | $SC_2H_4N(iPr)_2$ |

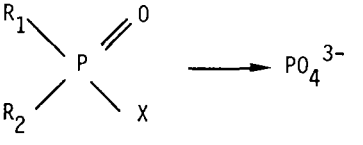
During the impact of nerve agents on the human body the active centre of the enzyme acetylcholinesterase is irreversibly phosphonylated. Once the enzyme is deactivated it can not fulfil its function anymore viz. the hydrolysis of acetylcholine, which plays an important role in the transfer of nerve stimuli. Consequently, the acetylcholine concentration is increased and the specific symptoms of poisoning appear.

## 2. PAST AND PRESENT DETECTION TECHNIQUES

Several methods of detection will be described in this section and examples of their applications will be given. However, this will be limited to chemical and biochemical methods.

### The "molybdenum blue" method

One of the oldest chemical methods for the detection of organophosphorous compounds is an extension of an ordinary phosphate detection method.[3] The organophosphorous compounds are destroyed oxidatively to orthophosphate by means of e.g. sulphuric acid or perchloric acid (Reaction scheme 1).



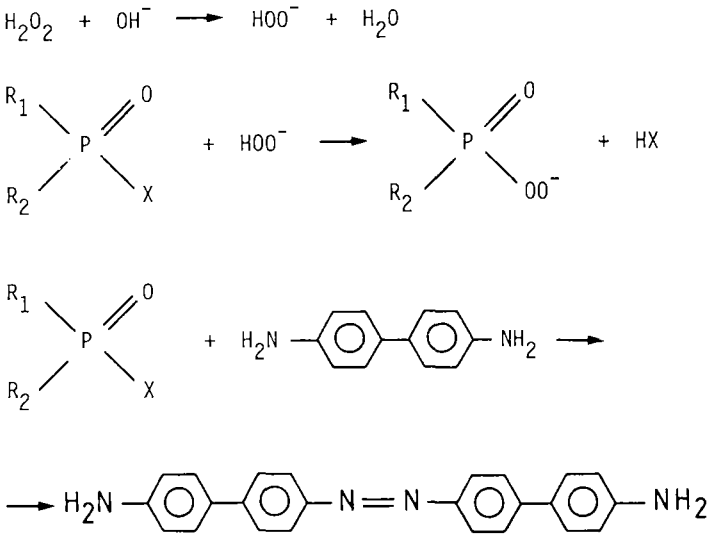
$$PO_4^{3-} + (NH_4)_6Mo_7O_{24} \longrightarrow H_3\left[P(Mo_3O_{10})_4\right] \xrightarrow{\text{Reductor}} \text{Blue colour}$$

**Reaction scheme 1** The molybdenum blue method.

The orthophosphate anion reacts with ammonium heptamolybdate in sulphuric acid to form a reddish phosphomolybdenic acid, which is then reduced to a blue coloured compound. As this method suffers from many interferences West *et al.*[4,5] modified it by adding o-dianisidine in the last step thus improving its sensitivity and selectivity. At the moment the molybdenum blue method is hardly in use anymore for the detection of organophosphorous compounds. A similar method for the detection of arsenic compounds is still being used.

### The Schoenemann reaction

The Schoenemann reaction[6] is based on the fact that peroxophosphonates do oxidize amines much easier than other peroxy ions (Reaction scheme 2).



**Reaction scheme 2** The Schoenemann reaction.

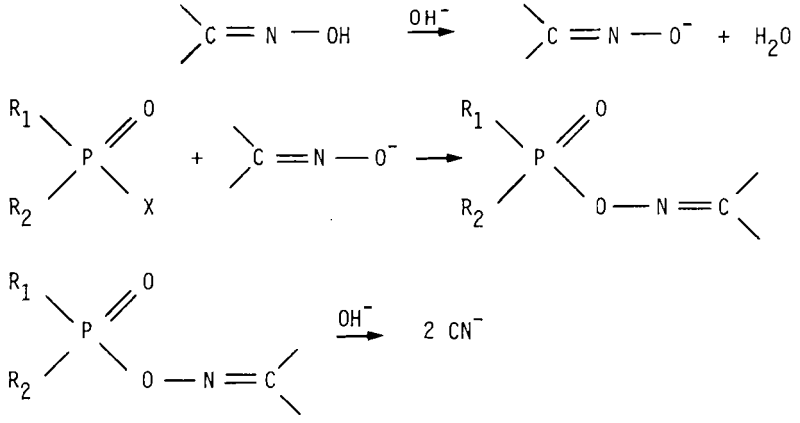
First, the organophosphorous compound reacts with hydrogen peroxide or sodium borate in an alkaline solution (pH 9–10). Then the peroxophosphonate reacts with a leuco-dye like benzidine or o-dianisidine to form an orange-brown coloured product. In certain modified reactions the amine is replaced by a precursor of a chemiluminescent compound (e.g. luminol)[7] or a fluorescent compound (e.g. indole).[8] Many organophosphorous compounds have been tested and it was shown that only labile P-X bonds (G-agents) give a positive reaction. Other ions that may be present or hydrolysis products of phosphonates do not interfere, and sometimes even enhance the luminescence.

The luminescent variant of the Schoenemann reaction finds practical application for civil and military purposes in portable, mobile and stationary laboratories.[9,10] The colorimetric variant of the Schoenemann reaction has been applied in so-called detection tubes. Air passes through a silica layer containing the dye. Then the peroxide solution is added from an internal breakable ampoule. Draeger (FRG) marketed such a detection tube for tabun and sarin (type Tabun-Sarin) having a detection limit of about 10 mg/m3. It

should be noted that these tubes have been replaced by detection tubes employing the enzymatic detection principle (type Phosphorsaureester) instead of the Schoenemann reaction.

## Electrochemical detection

An electrochemical method of detection is based on the fact that some organophosphorous compounds react with certain oximes like isonitrosobenzoyl acetone (IBA)[11] (Reaction scheme 3).



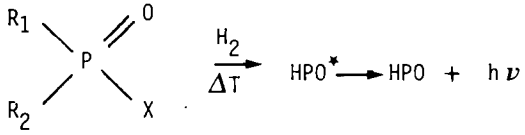
**Reaction scheme 3** Reaction of organophosphorous compounds and oximes.

The anion of the oxime reacts with the organophosphorous compound having a good leaving group X. In case of V-agents a reactive leaving group is introduced upon reaction with a silver-fluoride conversion filter. The intermediates yield cyanide ions via a Beckmann rearrangement in an alkaline solution. Upon reaction with IBA one cyanide ion yields two cyanide ions in return. The cyanide can be detected via a colorimetric reaction with p-nitrobenzaldehyde or can be determined electrochemically.

Based on this electrochemical method of detection a miniature chemical agent detector (ICAD) has been developed by Bendix (USA). The detector consists of a reusable electronic module (processor, audible alarm and warning light), and a disposable sensor module containing the battery power source and the sensor cells.

## Photometric detection

Normally there are no organophosphorous compounds in the atmosphere. Therefore a more or less selective method could be developed based on the detection of the phosphorus atom using flame emission.[12] When organophosphorous compounds are burnt in a hydrogen-rich flame excited HPO molecules are formed (Reaction scheme 4).



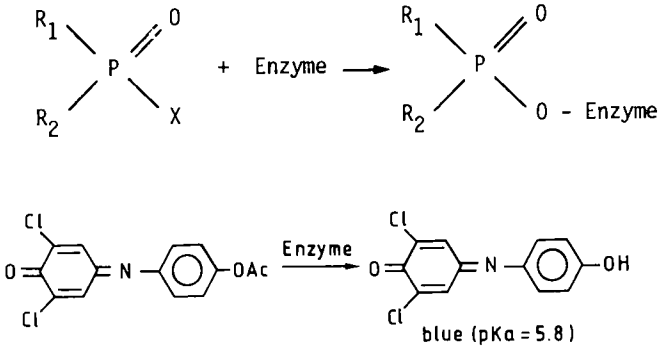

**Reaction scheme 4** Photometric detection.

When these excited molecules fall back to their ground state light is emitted in the range of 500–550 nm. Especially, the intensive band near 526 nm is characteristic for phosphorus and can be measured. In France an automatic warning system (Detalac) has been developed for military purposes. The hydrogen is generated in a disposable unit by reaction of aluminum and hydrogen fluoride. For civil use both a GC detector (Tracor) and an environmental monitor (Meloy) have been developed able to measure both phosphorus as well as sulphur.

## Detection based on enzyme inhibition effects

By far the most important method of detection at this moment is based on the enzyme inhibiting properties of certain organophosphorous compounds.[13] As such these detection devices can be regarded as first examples of biosensors. In these devices the naturally involved acetylcholine is replaced by a chromogenic substrate, like 2,6-dichloroindophenyl acetate (Reaction scheme 5).

Normally, the enzyme will catalyze the hydrolysis of the ester group of the substrate, which causes a distinct colour change from orange-red to blue. The difference in colour between the active and inhibited state of the enzyme can be observed visually or spectrophotometrically.




**Reaction scheme 5** The colorimetric version of the enzymatic detection reaction.



Based on this principle a number of simple, manually operated field tests (detection tubes, reagent papers) as well as automatic functioning monitors (warning equipment) have been developed. In the Netherlands the "button"-detector is marketed by Duphar BV. This coin-sized individual detector (Figure 1) consists of a plastic holder containing two separated air-permeable reagent papers, one impregnated with the enzyme and silica and the other with the substrate. Also, the detector contains a reservoir which releases the reagent solution when punctured. When air is drawn through the enzyme paper, the organophosphorous compounds are adsorbed on the silica. Then the counterparts of the "button"-detector are pressed together. The reagent solution is released, the reagent papers are wetted and at the same time pressed together initiating the enzymatic detection reaction. After two minutes the blue colour of the decomposed substrate can be clearly observed.

A similar test (water detection sticks) is available for checking the presence of cholinesterase inhibiting compounds in waste water streams. This test is produced by Rijling B.V. in the Netherlands. In automatic functioning monitors (designed as warning equipment) the residual activity of the enzyme can be determined either by a colorimetric reaction or electrochemically. In the Netherlands (Oldelft) the enzymatic reaction is performed on a continuous moving tape. The colour change is observed photometrically, followed by a conversion into audible or visible warning signals. In England (EMI Thorn) butylthiocholine is used as substrate. When it is decomposed by the immobilized enzyme the produced thiocholine

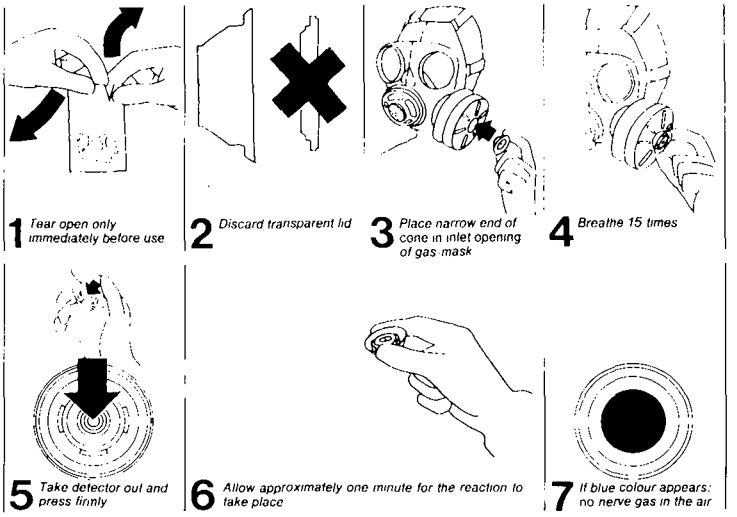
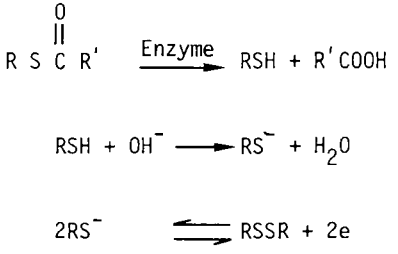



**Figure 1** The "button" enzymatic chemical nerve agents detector.

is measured electrochemically by anodic oxidation to form cholinedisulphide (Reaction scheme 6).




**Reaction scheme 6** The electrochemical version of the enzymatic detection reaction.

## Ion mobility detection

This detection technique is based on measuring the difference in mobility of various ions under influence of an electric field.[14,15] In the measuring cell an air stream passes a radioactive source (e.g.

63-Ni) generating ions like $H_3O^+$ or $H_2O_3^-$. Multiple collisions of these ions with the organophosphorous compounds cause the formation of charged ion clusters. Especially organophosphorous compounds are extremely efficient in forming such clusters. Also these clusters are heavier and therefore more stable than the ion clusters of other compounds normally occuring in the atmosphere. The charged clusters are now brought into a path with an electric potential gradient or an extremely tortuous path. The ions are separated by their difference in mobility (weight).

This technique is being applied in a number of alarm systems for nerve agents. In the USA the M43A1 chemical agent detector (Honeywell) uses a tortuous path. In England a portable and handheld chemical agent monitor (CAM) is developed (Graseby Dynamics) using the time of flight separator.



## 3. FUTURE TRENDS IN DETECTION METHODS

### Microsensors

In modern warfare the units tend to operate more dispersed. Individual detection devices will then become more and more important, requiring further automation and miniaturization of detection equipment, i.e. microsensors are to be developed.

The objectives to be met by the microsensors will be: small size, light weight, robust and low cost. With respect to the general performance characteristics a sensitive (1–10 ppb), fast responding (0–10 s), reversible and selective response of the sensor will be required. The detection principle must be readily adaptable to various toxic compounds and the equipment must be simple to operate and have a long shelf life (5–10 years).

In recent years many miniaturizing technologies have been investigated. Chemical Sensitive Semiconductor Devices (CSSDs) such as chemiresistors and CHEMFETs, optical wave guides and acoustic sensors.[16] King[17] introduced the use of bulk acoustic waves (BAW) with his Piezoelectric Sorption Detector. Wohltjen and Dessy[18] extended this detection principle to surface acoustic waves (SAW).

### Surface acoustic wave devices

Acoustic waves can be generated by applying an interdigital trans-

ducer on a piezoelectric substrate. When the crystal orientation of the substrate is properly chosen these waves propagate along the surface to the other transducer. Changes in the physical characteristics of the wave path will cause changes in the propagation velocity, which can be determined very sensitively and accurately by frequency measurement. When the wave path is covered with a so-called chemical interface (Figure 2) which reacts selectively and reversibly with the gas to be measured a SAW chemosensor can be realized.

In our laboratory a SAW-chemosensor for NO2 has been developed using metal-free and metallophthalocyanines as chemical interface.[19,20] A so-called dual delay-line configuration was used in order to compensate for non-specific effects. (Figure 2).



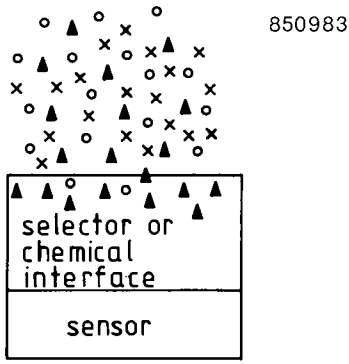



**Figure 2** Schematic presentation of a chemosensor, being a combination of a sensor and a selector or chemical interface.

The type of interaction between the gas and the chemical interface, the structure (morphology) of the interface material and the way it is attached to the surface of the sensor will determine the ultimate performance characteristics of the detection device.

When using the *absorption* phenomenon selectivity is determined only by slight differences in the partition coefficient between gas and chemical interface. Generally a poor selective sensor is obtained. In case of *adsorption* only weak interactions occur when the gas deposits on the interface material. The energies involved range from van der Waals' forces (0–10 kJ/mole) to acid-base interactions

(<40 kJ/mole). In case of *chemisorption* very strong interactions occur at the chemical interface. Chemical bonds are broken and other covalent or ionogenic bonds can be formed (energy per bond ~300 kJ/mole). As far as selectivity is concerned a specific chemical reaction or chemisorption is preferred. However, the gases are then strongly and often irreversibly bonded. So, for reversibility the weaker adsorptive interactions are preferred. Obviously these conflicting requirements need a compromise. Such a compromise can be found in the area of *coordination chemistry* or charge transfer complex formation (Figure 3).

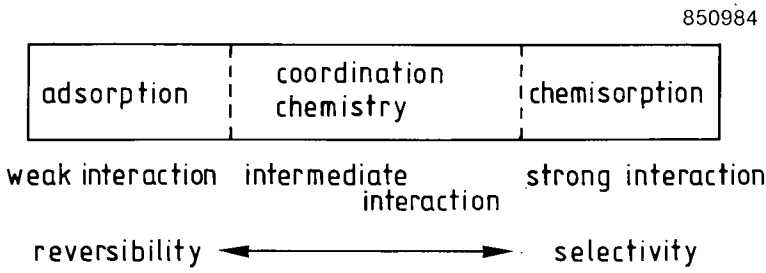



**Figure 3** Gas-solid interactions with respect to the selectivity and reversibility as some general performance characteristics.

In Table II the compounds are listed which have been suggested for the use as chemical interface. A division is made with respect to the expected physical or chemical interaction. It should be emphasized that these classifications are sometimes rather arbitrary. Often literature is not precise with regard to the information about the chemical interactions involved.

It can be concluded that three types of interaction could give rise to a more or less selective detection of organophosphorous compounds:

—Compounds containing metal ions like Cu(II) or Fe(III). Here coordination compounds are formed between the metal ion and the organophosphorous compounds. It was shown already that coordination compounds may act as a compromise between the weak and reversible adsorption and the strong and often irreversible chemical reaction.

**Table II** Types of chemical interfaces found in literature for sensors using gas/solid interactions

| Interaction/ chemical interface | Sensor | Organo P compounds (1) | Ref. |
|---|---|---|---|
| *Absorption* | | | |
| poly(vinylpyrrolidone) | BAW | DIMP | 21 |
| *Chemisorption* | | | |
| oxime coatings | BAW/electrode | (2), DDVP | 2,22,23 |
| 3-PAD (1) | BAW | DIMP, pesticides | 24 |
| 3-PAD + PVP (1) | BAW | DIMP | 21 |
| *Coordination* | | | |
| poly-amino Cu-comp. | CHEMFET/resistor | DIMP, DMMP | 25–28 |
| Fe(III)-chloride | BAW | DIMP | 29 |
| Au-, Ag-, Ni-compounds | BAW | DIMP | 30 |
| Cu(II)-PC(1) | resistor | DMMP | 21 |
| Cu(II) TMEDA/PVP(1) | BAW | DIMP | 31 |
| nitro PPA (1) | resistor | DIMP | 32 |
| *Biosensing* | | | |
| enzyme(3) | IR | general | 33 |

(1) DIMP = diisopropyl methylphosphonate
DMMP = dimethyl methyl phosphonate
DDVP dimethyl 2,2-dichlorovinylphosphonate
PC = phthalocyanine
3-PAD 1-n-octyl-3-(hydroxyiminomethyl) pyridinium iodide
TMEDA = tetramethylenediamine
PVP = poly(vinylpyrrolidone)
PPA = poly(phenylacetylene)
(2) all kinds of fluorophosphonates
(3) irreversible reaction

—Compounds containing oxime groups. However, they often react irreversibly with the organophosphorous compounds.

—Enzymes. They interact irreversibly with the organophosphorous compounds as well.

The common disadvantage of the examples mentioned in the literature is the way the chemical interfaces are bonded to the surface of the sensor i.e. physical attachment. Especially at elevated temperatures and in gas streams this method will cause serious stability problems. Therefore, we adhere to the concept of covalently bonded chemical interfaces on the surface of the chemosensor, which

will lead to smaller response times and improved stability as well as a more efficient relation between mass changes and the wave propagation properties. The search for suitable chemical interfaces for the detection of organophosphorous compounds, which can be immobilized at the surface of the sensor is in progress at our laboratory.

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