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22. Reviews by and/or discussions with Jerris Gieskes, James Bischoff, Michael Arthur, and Peter Lonsdale are gratefully acknowledged. This is contribution M-636 of the Scientific Department of Centre Océanologique de Bretagne.

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## Chemical Warfare Agents: Verification of Compounds Containing the Phosphorus-Methyl Linkage in Waste Water

Abstract. *The chemical analysis of the waste water from plants that manufacture chemicals is a nonintrusive method for confirming a suspected violation of the prohibition against the production of chemical warfare agents. The chemical structure of most nerve gases is related to that of methylphosphonic acid, whereas most organophosphorus pesticides have the phosphoric acid structure. On the basis of this characteristic difference, a procedure has been developed in which the presence of a nerve gas, its decomposition products, or its starting materials in waste water (Rhine River and Meuse River water) is reflected by the appearance of methylphosphonic acid as a breakdown product after hydrolysis. This acid is concentrated and converted into a volatile compound by methylation. After cleanup, the ester may be separated from related compounds by gas chromatography and is detected by means of a specific detector for phosphorus. The detection limit of nerve gases by this procedure is approximately 1 nanomole per liter of water. The scope and limitations of the method are discussed.*

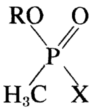
Since chemical warfare was initiated with the use of chlorine gas in World War I, a large number of candidate chemical agents has become available. Their characteristics vary considerably, and among them one finds irritants, vesicants, suffocating agents, paralyzants, psychochemicals, defoliants, and extremely toxic nerve gases. Recently a review on proven or alleged instances of the use of chemical warfare agents has been published (*1*).

As a result of the massive use of some of these agents during World War I, the Geneva Protocol of 1925 was established, prohibiting the (first) "use in war of asphyxiating, poisonous or other gases" (*2*, p. 69). Because of ambiguities in the text of the Protocol, the Secretary-General of the United Nations called upon all countries to reach agreement to halt the development, production, and stockpiling of all chemical (and biological) agents for purposes of war (*2*). Discussions on such a treaty started in the Conference of the Committee on Disarmament in Geneva in 1969.

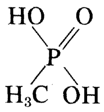
Many countries consider an adequate verification of such a production ban a prerequisite for the treaty. The main function of an effective verification system is to deter the production of chemical warfare agents, in particular the most dangerous. To achieve adequate deterrence, procedures are necessary to ensure that there is a reasonable probability that clandestine production will be detected. Clearly, the least intrusive verification methods are preferred.

A variety of methods has been proposed to confirm a suspected violation of the prohibition (*3*), including economic monitoring, satellite observation, and analysis of atmospheric, liquid, and solid waste samples. Among these, in addition to direct liquid waste analysis, the near-site inspection of river water downstream of chemical production plants showed particularly interesting possibilities.

In 1977 the Dutch delegation to the Conference of the Committee on Disarmament presented a working paper (*4*) on the verification of the presence of nerve gases, their decomposition products, or their starting materials in river water as a model for waste water. Because of their extreme toxicity, the nerve gases constitute the most serious threat among chemical warfare agents. Apart from very small quantities used in medicine these compounds do not seem to have any legitimate use. The nerve gases (**I**) are organophosphorus compounds and are structurally related to pesticides (**II**). Both types of compounds may be prepared in similar production plants. However, there is an important structural difference between these compounds. Most of the nerve gases are related to methylphosphonic acid (**III**), whereas most of the commercially available organophosphorus pesticides have phosphoric acid (**IV**) as their basic structure. A few experimental pesticides (*5-7*) are based on structure **III**.

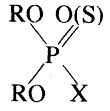


**I**

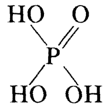


**III**

Examples of structure **I** include VX, in which R = $C_2H_5$ and X = $SCH_2CH_2N(i\text{-}C_3H_7)_2$, and Sarin, in which R = $i\text{-}C_3H_7$ and X = F.



**II**



**IV**

An example of structure **II** is parathion, in which R = $C_2H_5$, X = $OC_6H_4NO_2$-*p*, and P=O(S) becomes P=S.

The Japanese delegation to the Conference of the Committee on Disarmament has drawn attention (*8*) to the fact that the phosphorus-carbon bond is not cleaved under mild decomposing conditions. Moreover, Milly (*9*, p. 172) stated that, no matter what type of treatment is used on the discharge, it will be most unlikely that all characteristic components will be destroyed. Compounds containing phosphorus-methyl linkages, for example, will almost certainly persist and be identifiable for long periods after discharge. Gas chromatography in combination with specific phosphorus detection was proposed by the Japanese delegation as a suitable method for detecting organophosphorus compounds at very low concentrations.

The evaluation of a verification procedure, based on the above-mentioned considerations, is presented here. A more detailed description of the procedure is given in (*10*). Samples from the Rhine and Meuse rivers, both considered to be heavily polluted, were used as models for substantially diluted waste water downstream of chemical production plants. The compound VX was used as a representative of the nerve gases.

The first step in the verification procedure consists of the hydrolysis of the water sample. After a complete hydrolysis, most phosphorus-containing nerve gases will be converted to methylphosphonic acid, whereas organophosphorus pesticides will hydrolyze to phosphoric acid. In order to establish optimum conditions, we collected hydrolysis data for a number of organophosphorus compounds (*10*). These data indicated that nerve gases and pesticides hydrolyze completely to methylphosphonic acid and phosphoric acid, respectively, in 24 hours at *p*H 3 and 160°C.

After hydrolysis the water sample is passed through glass-fiber filters to remove solid particles (silt). The adsorption of methylphosphonic acid onto the solid particles in the river sample is negligible (as determined in experiments with $^{32}$P-labeled methylphosphonic acid).

A strong anion-exchange type resin (AG1) $N(CH_3)_3^{\oplus}$ ($HCOO^{\ominus}$ form) is used to adsorb the methylphosphonate anion from the hydrolyzed water sample. A simultaneous adsorption of other anions occurs, for example, chloride, sulfate, and phosphate, which may be present in excess when compared with the amount of methylphosphonate anion.

After the passage of the water sample, the resin is washed with methanol to remove the water together with some neutral and basic compounds present in the original water sample. The acids are eluted from the column with a hydrochloric acid–methanol solution. Between 75 and 100 percent of the methylphosphonic acid was recovered after evaporation by this procedure (as checked in experiments with $^{32}$P-labeled methylphosphonic acid).

Since methylphosphonic acid is not volatile enough to be gas-chromatographed, it is converted into the more volatile compound dimethyl methylphosphonate by treatment with diazomethane in diethyl ether solution (*11*). Other acids present in the water sample such as phosphoric and sulfuric acid (*12*) are methylated simultaneously, giving trimethyl phosphate and dimethyl sulfate, respectively. Ether and methanol are removed from the esterified sample by boiling under reflux in a Vigreux column. The yield from the esterification procedure is nearly quantitative (95 percent) as determined by gas chromatography.

At this stage, a cleanup procedure was introduced because the gas chromatographic separation of small amounts of dimethyl methylphosphonate from a 1000-fold excess of trimethyl phosphate proved to be insufficient and resulted in overlapping of the peaks. Moreover, dimethyl sulfate interfered seriously in the detection of dimethyl methylphosphonate, as demonstrated by experiments with Rhine River water samples doped with small quantities of VX (0.1 to 1 μg liter$^{-1}$).

A small silica gel column (*13*) removes most of the dimethyl sulfate and trimethyl phosphate from the methylated sample solution. The column is successively eluted with benzene, ethyl acetate, and methanol. The first milliliter of the methanol fraction contains dimethyl methylphosphonate. The recovery from this step of the procedure amounts to about 80 percent.

Dimethyl methylphosphonate and trimethyl phosphate are separated on a packed column in which Triton X-305 is used as the stationary phase. The column temperature is 150°C, and the injection volume is 5 μl. To prove unambiguously that the peak ascribed to dimethyl methylphosphonate is not due to the presence of a nonphosphorus compound in relatively high concentration, a thermionic detector was used in combination with a flame ionization detector. In the case of a nonphosphorus compound, a flame ionization detector will give a relatively high response.

Dimethyl methylphosphonate is identified by means of its retention index (*14*) (1451 ± 3 under the experimental conditions given above); trimethyl phosphate has a retention index of 1507 ± 1. The mean lowest amount of dimethyl methylphosphonate detectable proved to be 0.23 ng (range, 0.15 to 0.30 ng).

This detection limit corresponds to 248 ng of VX or about 1 nmole of a compound containing the phosphorus-methyl linkage per liter of water, being corrected for a mean recovery of 80 percent and an original water sample volume of 0.5 liter, which was concentrated during the complete verification procedure to a volume of 1 ml.

We checked the verification procedure by carrying out experiments with demineralized water and Rhine River water samples doped with varying quantities of VX (0.1 μg to 1 mg liter$^{-1}$). A recovery of about 80 percent dimethyl methylphosphonate was obtained. In case of high concentrations of VX in Rhine River water, the cleanup step was omitted.

Since 1975 a number of Rhine River and Meuse River samples have been taken to determine the background concentrations of compounds containing the phosphorus-methyl linkage. The mean background values expressed as the amounts of dimethyl methylphosphonate were 1 and 0.2 μg liter$^{-1}$, respectively. In addition to identification by gas chromatography, this compound was confirmed by mass fragmentography with a quadrupole gas chromatograph–mass spectrometer combination (*15*). The recovery obtained for Rhine River water samples doped with VX was accordingly corrected.

Our examination of the literature gives no indication that compounds containing the phosphorus-methyl linkage occur in nature. Therefore, it came as a surprise to us that a small amount of one or more of these compounds was also found in the samples of two relatively clean surface waters in the Netherlands [Maarsseveen (0.2 μg liter$^{-1}$) and Vlagtwedde (approximately 0.05 μg liter$^{-1}$)].

We believe that a positive identification of dimethyl methylphosphonate in the waste water (on the basis of its gas chromatographic retention index) constitutes sufficient evidence to warrant an inspection of the plant under consideration to obtain more definite proof of the violation of the prohibition. The use of more elaborate but less available techniques such as capillary gas chromatography–mass spectrometry, by which a superior resolution of the gas chromatographic peaks and a more clear-cut elucidation of the structures of the different peaks are obtained, might be advisable in later stages of the investigation.

For economic and environmental reasons stringent limitations have been placed on the waste discharge of chemical production facilities, for example, pesticide manufacturers. Notwithstanding this, a case study (*6, 16*) indicates that 0.01 to 0.1 per mil of the insecticide produced in such plants may be lost by way of the waste water. Data about the plants that produce nerve gases are scanty. In the United States a Sarin plant has been designed with a production capability of 12 tons per day, a scale that is comparable with the above-mentioned insecticide plant (*17*). Because of the extreme toxicity of nerve gases and for security reasons, it is reasonable to expect that the amount of these compounds in the discharge will not exceed 0.01 per mil.

This means that the nerve gas production facility may carry off 120 g of Sarin (or more probably an equivalent amount of its decomposition products) per day (*18*) into a waste stream with a flow rate of 7 liter sec$^{-1}$ (*9*, p. 171); this results in a Sarin concentration of about 200 μg liter$^{-1}$, which far exceeds the detection limit of the verification procedure (140 ng liter$^{-1}$) (*19*). Ultimately, the waste stream may flow into a river. In the case of river flows of 200 or 1000 m$^3$ sec$^{-1}$ (*20*), the concentration of Sarin or its degradation products in the water samples finally drops to about 7 or 0.7 ng liter$^{-1}$, respectively, which lie far below the detection limit. Within a few hundred meters downstream of the waste outlet, before achieving a complete mixing across the river, the concentration of the nerve gas will be above this limit (*21*).

To establish if a certain plant carries

off compounds containing the phosphorus-methyl linkage, it is necessary to compare a downstream sample with an upstream sample. The size of both samples must be such that a specified difference in terms of standard deviation will be found statistically significant according to the *t*-test with a chosen level of significance in a specified percentage of cases. With a level of significance of .05, a difference of 2 standard deviations will be found with a probability of .95 if both sample sizes are chosen to be seven or more (*22*). In our measurements we found that the standard deviation was about 12 percent of the determined value. This means that with a background value of 1.2 $\mu$g (the highest value found up to now) a downstream concentration of 0.29 $\mu$g will be detected under the above specified conditions; with a background of 0.5 $\mu$g, this concentration is 0.12 $\mu$g.

The commercially available pesticide Mecarphon (*7*)

$$[(CH_3O)CH_3P(S)SCH_2C(O)N(CH_3)COOCH_3]$$

will give rise to dimethyl methylphosphonate and will thus interfere in the verification procedure. In the case of the insecticide Dyfonate

$$[(C_2H_5O)C_2H_5P(S)SC_6H_5]$$

dimethyl ethylphosphonate will result. This compound (retention index, 1495 ± 1) is easily distinguished from dimethyl methylphosphonate (retention index, 1451 ± 3) during gas chromatographic analysis. The potential nerve gas Tabun

$$[(C_2H_5O)(CH_3)_2NP(O)CN]$$

and related compounds will not be detected upon application of this verification procedure because these compounds are derivatives of phosphoric acid and consequently yield trimethyl phosphate after hydrolysis and methylation.

Binary nerve gases are made by mixing two compounds during the delivery of the projectile to its target. For the nerve gases Sarin and Soman

$$[(CH_3)_3C(CH_3)CHOP(O)(CH_3)F]$$

methylphosphonic difluoride will probably be one of the binary components (*23*). This substance as well as Sarin and Soman can be detected with the proposed verification procedure. However, preliminary experiments indicated that the phosphorus-containing component QL

$$[(C_2H_5O)CH_3POCH_2CH_2N(C_3H_7)_2]$$

a possible precursor for binary VX (*23*), is not readily detected with the verification procedure described here.

The reported procedure for the verification of the presence of nerve gases, their decomposition products, or their starting materials in waste water gives a simple yes or no answer to the question of whether compounds related to these agents containing the phosphorus-methyl linkage are present or not. The method is sufficiently sensitive to give a positive indication even after extensive waste water purification and can be used even in heavily polluted water. In cases of strong dilution of the waste stream flowing into a river, the production of nerve gases may be masked.

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15. The gas chromatographic peak was scanned at three characteristic mass-to-charge values, 79, 94, and 109, which correspond to

$$(CH_3O)P(O)H^{\oplus}, (CH_3O)P(O)H(CH_3)^{\oplus\cdot}, \text{and } (CH_3O)_2P(O)^{\oplus}.$$

The peak intensity ratio was 6:4.4:1, which equals the result obtained with a reference sample of dimethyl methylphosphonate. Owing to the small amount, the intensity of the molecular ion was insufficient to permit scanning.
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19. The sensitivity of the method (1 nmole liter$^{-1}$) is equivalent to a detection of 140 ng of Sarin per liter.
20. The Meuse River at Liege, Belgium (1975) had a mean flow rate of 189 m$^3$ sec$^{-1}$ (range, 42 to 726 m$^3$ sec$^{-1}$). The mean flow rate of the Rhine River at Lobith, Netherlands (1975), was 2170 m$^3$ sec$^{-1}$ (range, 980 to 5341 m$^3$ sec$^{-1}$).
21. The formula $C_x = C_0Q_0/H(\pi DUx)^{1/2}$ was used, where $C_x$ is the maximum concentration at a distance $x$ from the waste outlet point, $C_0$ is the outlet concentration, $Q_0$ is the outlet flow, $H$ is the depth, $D$ is the diffusion coefficient $= 0.013HU$, and $U$ is linear flow. For both rivers it was assumed that $H = 2$ to 3 m and $U = 0.5$ to 1 m sec$^{-1}$.
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# Late Wisconsinan Sea Levels on the Southeast U.S. Atlantic Shelf Based on In-Place Shoreline Indicators

Abstract. *A new interpretation of late Pleistocene sea levels on the U.S. Atlantic continental shelf is based on in-place lagoonal and salt-marsh sediments obtained from vibra-cores. These data show sea levels during the last Wisconsinan transgression were about 30 meters shallower than is indicated by existing sea-level curves.*

Shoreline indicators obtained in vibracores permit new observations concerning Wisconsinan sea levels. Establishment of sea-level curves has generally been hampered by the difficulties involved in obtaining in-place shoreline indicators. The information reported here, obtained through vibra-coring, allows an evaluation of sea-level curves.

Pleistocene-Holocene sea-level curves commonly used for the U.S. Atlantic Coast are those of Milliman and Emery (*1*) and Curray (*2*), which differ significantly. A comparison of the two curves can be seen in Fig. 1. Milliman and Emery (*1*) have a much lower sea level from 16,000 to 11,000 years before present (B.P.) than Curray (*2*). There is up to 50 m difference in the depths between the two curves over portions of this time interval. From 25,000 to 18,000 years B.P. Milliman and Emery have a higher sea level than Curray. All curves are water depth–location curves because they are

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