Variation in the Toxicity of Arsenic Compounds to Microorganisms and the Suppression of the Inhibitory Effects by Phosphate

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The toxicity of potassium arsenate, as measured by retardation or inhibition of growth on solid nutrient media, showed wide variation among different fungi but was consistently reduced by the addition of large amounts of potassium phosphate, with both arsenic-sensitive and arsenic-tolerant fungi. *Poria monticola* was completely inhibited by 0.0025 M arsenate but was progressively less inhibited as the phosphate content of the medium increased and grew slowly at 0.04 M arsenate when 0.16 M KH$_2$PO$_4$ was added. *Cladosporium herbarum* showed 36% reduction in growth at 0.08 M arsenate in a low-phosphate medium, but when 0.01 M KH$_2$PO$_4$ was added, arsenate concentrations up to 0.64 M (at which the medium contains 4.8% As) caused no reduction in growth rate. Addition of phosphate also reduced the toxicity of potassium arsenite but not that of dimethyl sodium arsionate (sodium cacodylate). The counteracting effect of phosphate on arsenate toxicity was found to occur with every one of a wide variety of microorganisms tested. The author interprets the results as supporting the thesis that the fungitoxicity of arsenate is due to its competitive interference with phosphorus in oxidative phosphorylation and not to a reaction with the —SH groups of essential proteins. The latter mechanism is, however, probably operative with dimethyl sodium arsionate. The practical implications of the counter-inhibition phenomenon in laboratory investigations and standard tests of arsenical fungicides, in biochemical research, and in the commercial use of arsenical biocides are set out.

Arsenic compounds have been widely used as biocides both for research purposes and in agriculture, industry, and medicine because of their toxicity to microorganisms, plants, insects, and mammals. They are also used as selective enzyme inhibitors in biochemical research. Following the observation that performance of arsenic-containing wood preservatives could be adversely affected by incorporation of phosphate (D. F. McCarthy et al., *manuscript in preparation*), investigations were made into the antifungal action of various arsenic compounds and the effect of phosphates thereon.

**MATERIALS AND METHODS**

Two types of test were used. The first measured the growth of several fungi (mainly wood-destroying basidiomycetes) on agar containing various proportions of arsenic compounds and of phosphates. This test was intended to give a quantitative picture of the phosphate-arsenic interaction. The second measured the effect of phosphate on the growth-inhibition zones produced around an arsenical compound on agar plates seeded with the test organism. This test was used to determine whether this phosphate-arsenic interaction occurred with other types of microorganisms.

**Toxicity phial tests.** The arsenate-phosphate-nutrient media were prepared by autoclaving separately: (i) the appropriate mixtures of potassium phosphate (KH$_2$PO$_4$, pH 4.4) and potassium arsenate (produced by reacting As$_2$O$_3$·2H$_2$O with KOH to give an analogous pH of 4.4) and (ii) the nutrient medium. Five milliliters of each component was dispensed to screw-capped glass phials, which were then laid on one side to give an agar strip 90 mm long, 25 mm wide, and up to 3 mm deep. The final composition of the basal medium was: glucose, 1%; "Oxoid" mycological peptone, 0.2%; MgSO$_4$·7H$_2$O, 0.2%; thiamine hydrochloride, 0.002%; and agar, 1.5%. Similar procedures were used with KH$_2$PO$_4$ and potassium arsenite (As$_2$O$_3$, reacted with KOH to pH 4.4) and with KH$_2$PO$_4$ and
sodium cacodylate \([\text{Na}(\text{CH}_3)_2 \cdot \text{AsO}_3 \cdot 3\text{H}_2\text{O}]\) adjusted to pH 4.4 with 1 \(\text{m} \cdot \text{H}_2\text{SO}_4\).

Each toxicity phial was inoculated near the mouth with a 6-mm disc from a malt-agar colony of the test fungus, and incubated at 27 °C (see Fig. 2). The mean daily rate of linear growth was measured for duplicate phials, and, after 8 weeks, any inocula which had failed to grow were transferred to malt-agar plates to determine whether they were still viable (Tables 1–4).

**Seeded plate inhibition zone tests.** The surfaces of malt-agar plates (1.25% desiccated malt extract; 1.0% agar) were seeded uniformly with a thin film of spores, bacterial cells, or hyphal fragments, produced by blending a portion of a colony on a malt-agar plate in distilled water. A 6-mm disc of agar containing 0.4 \(\text{m} \cdot \text{sodium arsenate (equivalent to 2.7 mg of As)}\) was placed in the center of the plate, and a 6-mm agar disc containing 0.4 \(\text{m} \cdot \text{NaH}_2\text{PO}_4\) (1.1 mg of P) was placed near the edge of the plate 22 to 27 mm from the arsenical disc. The bioassay plates were usually held overnight at 5 °C to allow some diffusion of the arsenic and phosphate before growth commenced and then were incubated at 27 °C.

**TABLE 1.** Effect of potassium phosphate on fungitoxicity of potassium arsenate

| Test fungus | Amt of \(\text{KH}_2\text{PO}_4\) added | Linear growth rate (mm/day) on medium with potassium arsenate content of |
|-------------|-------------------------------------|---------------------------------------------------------------|
|             | 0.005 \(\text{m}\) | 0.02 \(\text{m}\) | 0.08 \(\text{m}\) |
| *Poria monticola* Murr. DFP 7522 | Nil | 4.2 | 0.0 | 0.0 | 0.0 |
| 0.01 \(\text{m}\) | 5.3 | 1.6 | 0.0 | 0.0 | 0.0 |
| 0.04 \(\text{m}\) | 5.2 | 2.6 | 0.6 | 0.0 | 0.0 |
| 0.16 \(\text{m}\) | 3.6 | 2.8 | 1.4 | 0.1 | 0.1 |
| *P. cocos* (Schw.) Wolf DFP 7281 | Nil | 12.2 | 0.0 | 0.0 | 0.0 |
| 0.01 \(\text{m}\) | 15.0 | 5.0 | 0.6 | 0.0 | 0.0 |
| 0.04 \(\text{m}\) | 15.6 | 15.0 | 8.0 | 1.4 | 0.0 |
| 0.16 \(\text{m}\) | 5.9 | 6.7 | 5.6 | 0.9 | 0.0 |
| *P. vaillantii* (DC. ex Fr.) Cke. DFP 4443N1 | Nil | 4.5 | 1.9 | 0.6 | 0.1 |
| 0.01 \(\text{m}\) | 4.8 | 4.4 | 3.5 | 1.4 | 0.0 |
| 0.04 \(\text{m}\) | 4.3 | 4.1 | 3.0 | 1.4 | 0.0 |
| 0.16 \(\text{m}\) | 2.8 | 2.9 | 2.8 | 1.2 | 0.0 |
| *Coniophora olivacea* (Fr. ex Pers.) Karst. DFP 1779 | Nil | 1.8 | 0.0 | 0.0 | 0.0 |
| 0.01 \(\text{m}\) | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.04 \(\text{m}\) | 2.6 | 0.4 | 0.0 | 0.0 | 0.0 |
| 0.16 \(\text{m}\) | 2.4 | 0.4 | 0.2 | 0.0 | 0.0 |
| *Lenzites trabea* (Pers.) Fr. DFP 7520 | Nil | 4.6 | 0.4 | 0.1 | 0.0*a |
| 0.01 \(\text{m}\) | 3.8 | 3.6 | 2.7 | 0.8 | 0.0 |
| 0.04 \(\text{m}\) | 3.7 | 3.0 | 3.0 | 2.4 | 0.0 |
| 0.16 \(\text{m}\) | 2.8 | 2.4 | 1.2 | 0.8 | 0.0 |

*a Inoculum still viable after 8 weeks.

**TABLE 2.** Effect of potassium phosphate on toxicity of potassium arsenate to arsenic-tolerant fungi

| Test fungus | Amt of \(\text{KH}_2\text{PO}_4\) added | Linear growth rate (mm/day) on medium with a potassium arsenate content of |
|-------------|-------------------------------------|---------------------------------------------------------------|
|             | 0.08 \(\text{m}\) | 0.16 \(\text{m}\) | 0.32 \(\text{m}\) | 0.64 \(\text{m}\) |
| *Poria vaillantii* (DC. ex Fr.) Cke. DFP 4443N1 | Nil | 4.8 | 0.0 | 0.0 | 0.0 |
| 0.01 \(\text{m}\) | 4.7 | 1.0 | 0.6 | 0.0 | 0.0 |
| 0.04 \(\text{m}\) | 4.4 | 1.4 | 0.6 | 0.1 | 0.0 |
| *Scopulariopsis brevicaulis* Bainier DFP 11821 | Nil | 1.2 | 1.0 | 0.9*a | 0.0*a | 0.0*a |
| 0.01 \(\text{m}\) | 1.4 | 3.6 | 3.6 | 2.3 | 0.9 |
| 0.04 \(\text{m}\) | 1.6 | 3.6 | 3.4 | 2.6 | 1.0 |
| *Cladosporium herbarum* (Pers.) Link DFP 0345 | Nil | 1.1 | 0.7 | 0.7 | 0.6 | 0.5 |
| 0.01 \(\text{m}\) | 1.3 | 1.4 | 1.4 | 1.5 | 1.4 |
| 0.04 \(\text{m}\) | 1.4 | 1.6 | 1.6 | 1.5 | 1.6 |

*a Inoculum still viable after 8 weeks.
soon as an inhibition zone around the arsenical disc could be detected, the radius of inhibition was measured in the direction of the phosphate disc and also in the diametrically opposite direction. These measurements and the ratio between them are given in Table 5. Where the organism was highly tolerant of arsenic, the inhibition zone would be small or absent and the test would be repeated with one, or even three, 12-mm arsenical discs (10.7 or 32 mg of As). With a very susceptible organism, all growth might be inhibited and the test would be repeated with a 6-mm disc of 0.1 mg sodium arsenate agar (0.7 mg of As). Since results are obtained by comparing different radii of inhibition within the one petri dish, such methodological variations do not invalidate the conclusions.

### RESULTS

The results in Tables 1 and 2 show that the addition of potassium phosphate had some influence on the arsenate tolerance of all fungi tested, but that the nature of this influence varied widely with the fungi.

With *Poria monticola* and some other arsenic-sensitive fungi (5), there was a slow but sustained increase in arsenate tolerance as phosphate concentrations increased, assuming

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**Table 3. Effect of potassium phosphate on fungitoxicity of potassium arsenite**

| Test fungus         | Amt of KH$_2$PO$_4$, | Linear growth rate (mm/day) on medium with potassium arsenite concentration of |
|---------------------|----------------------|---------------------------------------------------------------------------------|
|                     | added                | Nil          | 0.0006 M       | 0.0025 M       | 0.01 M         | 0.04 M         |
| *Poria monticola* Murr. |                     |             |                 |                 |                 |                 |
| DFP 7522            | Nil                  | 4.6         | 2.6            | 0.9            | 0.1            | 0.0            |
|                     | 0.01 M               | 5.4         | 5.0            | 3.6            | 1.7            | 0.0            |
|                     | 0.04 M               | 5.4         | 4.8            | 4.0            | 1.8            | 0.0            |
| *P. vaillantii* (DC. ex Fr.) |                 |             |                 |                 |                 |                 |
| Cke.                | Nil                  | 4.8         | 4.6            | 3.7            | 1.0            | 0.1            |
|                     | 0.01 M               | 4.8         | 4.9            | 4.2            | 2.4            | 0.4            |
|                     | 0.04 M               | 4.4         | 4.4            | 3.8            | 2.6            | 0.5            |
| *Coniophora olivacea* (Fr. ex Pers.) |                |             |                 |                 |                 |                 |
| Karst.              | Nil                  | 2.0         | 0.0            | 0.0            | 0.0            | 0.0            |
|                     | 0.01 M               | 2.2         | 0.2            | 0.0            | 0.0            | 0.0            |
|                     | 0.04 M               | 2.0         | 0.4            | 0.0            | 0.0            | 0.0            |
| *Trametes versicolor* (L. ex Fr.) |                |             |                 |                 |                 |                 |
| Lloyd               | Nil                  | 6.5         | 2.6            | 1.0            | 0.0            | 0.0            |
|                     | 0.01 M               | 4.8         | 3.2            | 2.2            | 1.2            | 0.0            |
|                     | 0.04 M               | 4.9         | 3.3            | 2.0            | 1.2            | 0.0            |

**Table 4. Effect of potassium phosphate on fungitoxicity of dimethyl sodium arsenate (sodium cacodylate)**

| Test fungus         | Amt of KH$_2$PO$_4$, added | Linear growth rate (mm/day) on medium with a dimethyl sodium arsenate content of |
|---------------------|---------------------------|---------------------------------------------------------------------------------|
|                     |                           | Nil          | 0.0025 M       | 0.01 M         | 0.04 M         | 0.16 M         |
| *Poria monticola* Murr. |                     |             |                 |                 |                 |                 |
| DFP 7522            | Nil                      | 4.0         | 1.5            | 1.2            | 0.2            | 0.0            |
|                     | 0.01 M                   | 5.8         | 2.4            | 0.8            | 0.0*           | 0.0            |
|                     | 0.04 M                   | 5.6         | 2.2            | 1.2            | 0.4            | 0.0            |
| *P. cocos* (Schw.) Wolf |                     |             |                 |                 |                 |                 |
| DFP 7281            | Nil                      | 13.4        | 4.4            | 2.8            | 0.0            | 0.0            |
|                     | 0.01 M                   | 15.9        | 6.4            | 3.0            | 0.0            | 0.0            |
|                     | 0.04 M                   | 17.2        | 4.3            | 2.6            | 0.0            | 0.0            |
| *P. vaillantii* (DC. ex Fr.) |                 |             |                 |                 |                 |                 |
| Cke.                | Nil                      | 5.0         | 4.6            | 3.6            | 0.4            | 0.0*           |
|                     | 0.01 M                   | 4.9         | 4.5            | 3.7            | 0.4            | 0.0*           |
|                     | 0.04 M                   | 4.5         | 4.2            | 3.4            | 0.3            | 0.0*           |
| *Coniophora olivacea* (Fr. ex Pers.) |                |             |                 |                 |                 |                 |
| Karst.              | Nil                      | 2.2         | 0.5            | 0.4            | 0.0            | 0.0            |
|                     | 0.01 M                   | 2.2         | 0.6            | 0.4            | 0.0            | 0.0            |
|                     | 0.04 M                   | 2.0         | 0.8            | 0.5            | 0.0            | 0.0            |
| *Trametes versicolor* (L. ex Fr.) |                |             |                 |                 |                 |                 |
| Lloyd               | Nil                      | 5.8         | 6.2            | 4.0            | 0.0            | 0.0            |
|                     | 0.01 M                   | 5.2         | 6.6            | 4.0            | 0.0            | 0.0            |
|                     | 0.04 M                   | 5.6         | 6.4            | 3.9            | 0.0            | 0.0            |

* Inoculum still viable after 8 weeks.
TABLE 5. Effect of phosphate on radius of inhibition of sodium arsenate on seeded plates

| Test organism                        | DFP no. | Radius of inhibition (mm) | Ratio of A:B |
|--------------------------------------|---------|---------------------------|--------------|
|                                      |         | A (away from phosphate plug) | B (towards phosphate plug) | |
| Basidiomycetes                       |         |                           |              | |
| Coniophora olivacea (Fr. ex Pers.) Karst. | 1779    | 29                        | 17           | 1.7:1 |
| Fomes annosus (Fr.) Karst.           | 10052A  | 26                        | 10           | 2.6:1 |
| Fusarium convulvulaceum (Pers.) G H Cunn. | 13585   | 26                        | 7            | 3.7:1 |
| Poria cocos (Schw.) Wolf             | 7281    | 18                        | 6            | 3.0:1 |
| P. monticola Murr.                   | 7522    | 26                        | 16           | 1.6:1 |
| P. vaillantii (DC. ex Fr.) Cke.      | 4443N1  | 25                        | 10           | 2.5:1 |
| Poria sp.                            | 3112    | 26                        | 15           | 1.7:1 |
| Trametes lilacina-gilva (Berk.) Lloyd | 1109    | 31                        | 16           | 1.9:1 |
| T. versicolor (L. ex Fr.) Lloyd      | 7521    | 22                        | 10           | 2.2:1 |
| Xylobolus frustulatus (Pers. ex Fr.) Boidin | 10375   | 30                        | 10           | 3.0:1 |
| Lower fungi                          |         |                           |              | |
| Acremoniella sp.                     | 7467    | 22                        | 5            | 4.4:1 |
| Aspergillus niger van Tiegh.         | 401     | 35                        | 17           | 2.1:1 |
| Chaetomium globosum Kunze ex Fr.     | 6400    | 16                        | 4            | 4.0:1 |
| Cladosporium herbarum (Pers.) Link   | 5345    | 22                        | 3            | 7.3:1 |
| Fusarium solani (Martius) Appel & Wollenweber | 4442   | 29                        | 19           | 1.5:1 |
| Mucor microsporus Namyslowski        | G552    | 35                        | 23           | 1.5:1 |
| Penicillium spinulosum Thom          | 8333    | 17                        | 8            | 2.1:1 |
| Actinomycete                         |         |                           |              | |
| Streptomyces griseus Waksman & Henrici | G299   | 26                        | 12           | 2.2:1 |
| Bacteria                             |         |                           |              | |
| Bacillus subtilis (Ehrenberg) Cohn   | G2      | 24                        | 12           | 2.0:1 |
| Pseudomonas aeruginosa (Schroeter) Migula | G133  | 20                        | 6            | 3.3:1 |
| Alga                                 |         |                           |              | |
| Chlorella pyrenoidosa Chick          | 13879   | 30                        | 5            | 6.0:1 |

*Unless otherwise noted, As was supplied as a 6-mm diameter disc of 0.4 M NaH$_2$AsO$_4$ and P as a 6-mm diameter disc of 0.4 M NaH$_2$PO$_4$.

* A 6-mm disc of 0.1 M NaH$_2$AsO$_4$.

* Three 12-mm discs of 0.4 M NaH$_2$AsO$_4$ in a pile.

* A 12-mm disc of 0.4 M NaH$_2$AsO$_4$.

that tolerance is best assessed by growth on arsenate-containing medium as a proportion of growth on similar arsenic-free medium (Table 1, Fig. 1A). [In some cases, the absolute rate of growth decreased at the 0.16 M level of KH$_2$PO$_4$ because of a slight inhibitory effect of the phosphate itself at this level, supporting the suggestion of Cochrane (3) that the upper limit for satisfactory use of phosphate buffers in fungal nutrient studies is 0.20 M.]

A different pattern was shown by most fungi which are tolerant of arsenate, e.g., *P. vaillantii*. Here the initial addition of phosphate produced an immediate large increase in tolerance which was not further increased by additional phosphate (Tables 1, 2; Fig. 1B, C). Tables 1–4 show that, even for arsenic-sensitive fungi, the results cannot be explained simply in terms of phosphorus-arsenic ratios.

Although potassium arsenite had been expected to be appreciably more toxic than the arsenate and had therefore been tested at lower concentrations, the results in Table 3 show that the two salts were of approximately the same toxicity and that the counteracting effect of the phosphate applied to arsenite also. Sodium cacodylate (Table 4; Fig. 1D) was less toxic than potassium arsenate or arsenite, but its toxicity was not counteracted by phosphate as that of the inorganic salts had been.

For all three toxicants, those inocula which had failed to grow were almost all nonviable at the end of the test, the major exception being those of *Scopulariopsis brevicaulis*, which were
Fig. 1. Effect of added phosphate on linear growth of test fungi on arsenic-containing agar. (A) Gradual counteracting effect of $\text{KH}_2\text{PO}_4$ against $\text{KH}_2\text{AsO}_4$ with arsenic-susceptible Poria monticola; (B) immediate counteracting effect of $\text{KH}_2\text{PO}_4$ against $\text{KH}_2\text{AsO}_4$ with arsenic-tolerant P. vaillantii; (C) complete tolerance of Cladosporium herbarum of $\text{KH}_2\text{AsO}_4$ on addition of $\text{KH}_2\text{PO}_4$; (D) absence of any effect of $\text{KH}_2\text{PO}_4$ against dimethyl sodium arsonate with Trametes versicolor.
still viable after 8 weeks of dormancy on high concentrations of arsenate (Table 2).

In the screening tests carried out in seeded petri dish cultures, distortion by a phosphate disc of the normally circular inhibition zone surrounding an arsenical disc on a plate uniformly seeded with the test organism gave clear evidence of a counteracting effect, although the appearance of the distortion varied greatly with the susceptibility and growth rate of the organism. Typical patterns of distortion are shown in Fig. 2. The quantitative measurements of the amount of distortion for each organism tested (Table 5) show that the radius of inhibition from the arsenical plug was reduced by phosphate with each of the varied microorganisms studied. (No distortion of the inhibition zone occurred when potassium sulfate was substituted for potassium phosphate.)

**DISCUSSION**

The results of the arsenate phial tests show that the ability of the fungi tested (arsenic-tolerant and arsenic-sensitive) to grow on an arsenate-containing medium was greatly enhanced by the addition of phosphate in relatively large amounts. The basal medium it-

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**FIG. 2.** Effect of agar plug containing NaH$_2$PO$_4$ (1.1 mg of P) on inhibition zone around central plug containing NaH$_2$AsO$_4$ (2.7 mg of As) on malt agar plates seeded with: (A) Trametes versicolor, an arsenic-sensitive basidiomycete; (B) Penicillium spinulosum, an arsenic-tolerant deuteromycete; (C) Mucor microsporus, a highly arsenic-sensitive phycomycete; (D) Streptomyces griseus, an arsenic-sensitive antinomycete.
self, although phosphorus-deficient, was not phosphorus-free, since the “Oxoid” mycological peptone used contained phosphate equal to 1.4% P₂O₅ (8), equivalent to 0.0004 m KH₂PO₄ in the final medium, as compared with an optimum concentration of 0.001 to 0.003 m cited by Cochrane (3).] The tolerance of Cladosporium herbarum exceeded even that found by Challenger (2), who reported that this fungus can tolerate arsenic concentrations of 2%, but not of 4% arsenic in solution. When 0.01 m phosphate was present, the growth of the fungus in the present tests was not affected in any way by a concentration of 4.8% arsenic in agar.

The consistent occurrence of a counteracting effect of phosphate on arsenate toxicity for all organisms tested, including the alga Chlorella pyrenoidosa, suggests that it affects some universal metabolic process. It would be interesting to know whether a similar effect occurs in the use of inorganic arsenicals as insecticides, herbicides, mammalian poisons, and enzyme inhibitors.

The toxicity of arsenical compounds to fungi has usually been ascribed to the reaction of arsenoxides with the sulphhydril groups of vital enzymes or other cell constituents [cf. Cochrane (3), Horsfall (6), and Torgeson (10)]. For animal cells, on the other hand, it has been postulated by Webb (11) and O’Brien (7) that arsenates interfere in oxidative phosphorylation, with the occurrence of arsenolysis instead of phosphorolysis, preventing the production of adenosine triphosphate. In this case, excess supplies of phosphate could be expected to interfere competitively with arsenolysis. The results described in this paper strongly suggest that the toxicity of arsenates to fungi may also be due to arsenolysis and not, as suggested in the literature cited, to blocking of sulphhydril groups.

Regarding the completely different results obtained with dimethyl sodium arsenate, Webb suggests (p. 596 of reference 11) that pentavalent organic arsenicals such as this produce their effects only after reduction to the trivalent form and that, like arsenite, they act mainly by reaction with —SH groups, especially with vicinal —SH groups. The most potent inhibition is caused by a reaction of the type:

$$\text{R - As} + \text{O} + \text{HS} \rightarrow \text{R - As} + \text{S}$$

where E is an enzyme possessing vicinal —SH groups. If this is the mechanism of action of sodium cacodylate, one would not expect the phosphate to have any counteracting effect.

The results obtained with potassium arsenite are more difficult to explain. Since its toxic effect was also definitely counteracted by phosphate, though perhaps not so markedly as that of the arsenate, one could postulate that it also has an arsenolytic effect. This is supported by O’Brien (7) for insects (although he implies that the major effect is by reacting with —SH groups); but Webb states (p. 658 in reference 11) that arsenite has little effect on oxidative phosphorylation. Arsenite can be oxidized to arsenate in vivo (p. 603 in reference 11), and this possibly could explain the discrepancy.

Regardless of the mechanism of this “antidoting” effect, its existence has important implications both in research and in the practical use of arsenical biocides. In any investigations involving bioassays of arsenical compounds or their use as enzyme inhibitors, phosphate buffers should be used with caution. For example, the British standard method for bioassay of wood preservatives against soft rot fungi (1) and the Australian standard method for measuring fungus resistance of materials (9) both make use of an agar medium containing approximately 0.03 m potassium phosphate; this is well above the level likely to be encountered by most materials in service, and the result could give a misleading assessment of the likely value of arsenical fungicides (4) and possibly of other fungicides. Similar media are used throughout the world for research purposes and for legal specifications and have recently been recommended by the International Standards Organization for tests of mold resistance. Since the dangers of using those high-phosphate media clearly are not suspected, a preliminary note (4) was published at an early stage of this investigation to warn other workers in this bioassay field. It is also possible that the commercial use of arsenical biocides may be ineffective in applications where high concentrations of phosphates are encountered, as in some agricultural soils, in sewage effluents, or in wood treated with fire retardants.

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