Studies on the Bioaccumulation and Microbial Degradation of 2,3,7,8-Tetrachlorodibenzo-p-dioxin

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While the problem of pesticidal contamination of the environment is far from being solved, considerable useful information has emerged from the research efforts made by many scientists in recent years.

First, we now know by experience that the chemicals that cause environmental problems are the ones which are extremely persistent in nature, biologically active, and easily concentrated in biological systems. Compounds which lack any of the above qualifications usually do not play any significant role in pesticidal pollution no matter how acutely toxic they are. The above analysis becomes more important, when one considers other aspects of pesticidal pollution. For instance, we are concerned about only biological effects in considering pollution, with particular emphasis on the effects on non-target organisms.

In the case of polychlorinated dibenzo-p-dioxins, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the question of bioactivity is indisputable, as it is one of the most toxic compounds known to occur as a pesticidal impurity (1–3). Its chemical stability is also questionable. Thus the central question of its hazard to the environment must be studied from the viewpoint of bioconcentration in various ecosystems.

Published data on environmental fate of chlorodibenzo-p-dioxins are scarce at present. Zitko (4), for instance, could not find residues of dioxins in several aquatic animals at detection limits of 0.01–0.04 µg/g. Isensee and Jones (5), studied absorption and translocation of TCDD and its 2,7-dichloro analog in root and foliage, and concluded that dioxins are neither readily picked up from the soil residues nor translocated into foliage, at least in the case of soybeans and oats.

Studies by Kearney et al. (6) indicate that the average TCDD remaining after weathering in soil for 1 yr is of the order of 50–60% at all concentrations tested (1 to 100 ppm), while the actual field survey (7) on the test areas with Agent Orange (1962–1970) revealed that much less TCDD residues remaining in soil than is expected from the above figure.

In the study reported herein we have made efforts to measure the degree of bioaccumulation of TCDD in relation to well established pesticides by using several model ecosystems. The data are still preliminary, in that several model ecosystems are still being compared for their relative merits in assessing the actual impact of pesticides in

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nature. The data obtained have been, however, useful in assessing the relative tendency of a pesticide in comparison with other pesticides.

Materials and Methods

Microbial Degradation of TCDD

Microbes—Approximately 100 microbial strains which have previously shown the ability to degrade persistent pesticides were screened for their ability to degrade TCDD.

Procedure—Screening was carried out and the metabolic products were examined by thin-layer chromatography (TLC) by the method of Matsumura and Boush.(8)

Translocation of Pesticides

Procedure—The pesticides (0.1 μmole each) were deposited on 1 g of clean sea sand, which was placed on a column of sandy loam type soil. Water was then slowly dropped onto the surface of the sand at a rate of approximately 2 ml/hr. The water and sections of soil were extracted with chloroform.

Bioaccumulation of Pesticides

Animals—Three groups of invertebrates were used for the pesticide accumulation study: Ostracoda species, Artemia salina, and Aedes aegypti larvae, and one fish species, northern brook silverside, Laludesthes sicculus.

Pesticides—Four pesticides were selected from representative groups of important compounds: dioxin (TCDD), DDT, γ-BHC, and zectran. All compounds were 14C-labeled in the benzene rings.

Procedure—Three model ecosystems were used to study bioaccumulation.

In model I, the pesticides (5 and 10 pmole) dissolved in a solvent were added directly to water along with the primary food organism, such as algae and yeast, and this mixture was then added to the aquarium containing the invertebrate test organisms.

In model II, the pesticides (20 pmole) were deposited on the inner surface of the glass container by evaporating the solvent to form a thin film. The primary food organism were grown in the container for 24 hr and then transferred along with the culture media to the aquarium containing the test invertebrate organism.

In model III, the pesticides (5 and 10 pmole) were deposited on 1 g of sand and the solvent evaporated to form a thin film on the surface of the sand particles. The sand was added to the test aquarium containing invertebrates and/or fish.

In all cases the test organisms were maintained in the aquarium at room temperature (24°C), except for the fish cultures which were maintained at 12°C. Test organisms were either homogenized in counting solution or carbonized (Model 300 Packard Tri-Carb Oxidizer), and the amount of 14CO2 measured. Measurements of the amount of labeled material in the water, primary food organism, and on sand and glass surfaces were made by extracting with chloroform. All studies were short-term (4–7 days), in small volume containers (200 ml).

Results and Discussion

As shown in Table 1, the extent of translocation of TCDD from the sand to the organic soil layer is extremely small. Virtually no TCDD was found to leach out from the column. The mobility of TCDD in soil, therefore, must be considered much less than that of DDT. Thus, the mode of translocation of TCDD in the environment would be limited to movement of soil particles or dust-carried dispersion and biological transfer (but not plant-mediated transfer), particularly in aquatic environments.

As for the microbially mediated degradation of TCDD, our current survey indicates that such capabilities are rather rare in nature. Approximately 100 microbial strains in which the ability to degrade persistent pesticides has been previously demonstrated were screened for this purpose. Among them, only five strains showed some ability to degrade this compound (Fig. 1). We have not been able to manipulate cultural conditions
to increase the rate of degradation of TCDD in any of the microorganisms so far.

In studying the extent of biological transfer of TCDD, three different model systems were devised. In model system I, pesticides in acetone were introduced directly into water along with the primary food organisms. In model system II, pesticides were applied to the inner surface of a glass container, and the primary food organisms were grown in the container for 24 hr and were transferred to the aquarium. In model III, pesticide-coated sands were placed directly in the aquarium containing the test organisms.

In the model I experiment (Table 2), DDT behaved quite differently from other pesticides, showing high degrees of affinity to each test organism, in close agreement with the phenomenon actually observed in nature. Although this model system is simple and appears to offer a quick straight-

**Table 1. Vertical translocation of pesticides from sand to organic soil.**

| Pesticide content, % | Dioxin b | DDT b | Zectran b |
|----------------------|----------|-------|-----------|
| Top sand             | 90.41    | 65.01 | 0.07      |
| 0–0.5 cm             | 7.52     | 30.75 | 0.06      |
| 0.5–1.0 cm           | 1.04     | 3.51  | 0.08      |
| 1.0–1.5 cm           | 0.50     | 0.55  | 0.05      |
| 1.5–2.0 cm           | 0.26     | 0.26  | 0.06      |
| 2.0–2.5 cm           | 0.18     | 0.19  | 0.06      |
| Water eluate c       |          |       |           |
| 1st 50 ml            | 0.12     | 0.06  | 49.4      |
| 2nd 50 ml            | 0.08     | 0.04  | 17.6      |
| 3rd 50 ml            | 0.09     | 0.02  | 29.1      |

*10 × 1.5 cm glass column.

b Pesticide introduced: 0.1 μmole each (33.8 μg for dioxin, 35.5 μg for DDT, and 22.2 μg for Zectran).

c Water eluted per day, 50 ml.

forward answer to the general tendency of pesticidal accumulation by biological systems, it has one weakness, i.e., that one is forced to work above the limit of water solubility of some of the compounds. TCDD for instance was measured at a level 100 times its water solubility. Also the extent of direct pick-up due to partitioning and food intake is uncertain. In the model II experiment, where only the portion of pesticide picked up by the primary food organisms and the media were introduced into the test aquarium, the levels of total pick-up were further reduced in the case of TCDD (but not DDT) (Table 3).

To circumvent the problem of solubility, the model III system was devised. In this way, only that portion of pesticide that is soluble should be present in water at any time. The results shown in Table 4 indicate that the rate of TCDD pick-up is extremely low in brine shrimp and fish under the experimental conditions. Mosquito larvae, which are bottom feeders, showed a surprising rate of TCDD pick up. The reaction is not at its maximal rate, since further increase in the level of the pesticide apparently increases the pick up by the larvae. Also noted is the difference between the bioconcentration pattern in fish as compared to other invertebrates. γ-BHC, in particular,
shows high degree of concentration in fish. To study the effects of food consumption, the same test was repeated in the presence of mosquito larvae.

As expected, the level of TCDD (Table 5) in the fish increased in the presence of mosquito larvae, which are the best concentrators of TCDD among the organisms tested. On the other hand, the levels of other pesticides did not significantly change, indicating that the route through ingestion of mosquito larvae does not represent the major source of uptake in these pesticides.

It is apparent from these data that the reaction of biological concentration is greatly influenced by the external conditions and the design of the experiment, the physical and biological nature of the organisms, and by chemical characteristics of the pesticides. To facilitate understanding of the role of chemical nature of pesticides in determining the rate of bioconcentration, a comprehensive list has been prepared to illustrate their important properties (Table 6).

It can be seen here that general tendencies of bioaccumulation in invertebrate species follow closely the trend of the partition coefficients. In model II experiments, however, the values for TCDD come much lower than expected from this rule. Thus it is likely that water solubility (and solvent solubility) must play an important role where the initial pick-up is the rate-limiting factor.

It is apparent that species-specific factors play a much more important role than once

| Test organisms (primary food) | Pesticide | Original concentration in water, ppb | Final concentration found in test organisms, ppb | Concentration factor |
|-------------------------------|-----------|--------------------------------------|-------------------------------------------------|----------------------|
| Daphnia (algae)               | Dioxin    | 32.4                                 | 1,592                                           | 49                   |
|                               | DDT       | 35.8                                 | 44,164                                          | 1234                 |
|                               | Zectran   | 22.2                                 | 1,969                                           | 89                   |
| Ostracod (algae)              | Dioxin    | 32.4                                 | 7,069                                           | 218                  |
|                               | DDT       | 35.8                                 | 50,771                                          | 1418                 |
|                               | Zectran   | 22.2                                 | 7,265                                           | 327                  |
| Brine shrimp (yeast)          | Dioxin    | 16.2                                 | 1,956                                           | 121                  |
|                               | DDT       | 17.9                                 | 12,336                                          | 689                  |
|                               | γ-BHC     | 14.7                                 | 2,688                                           | 183                  |
|                               | Zectran   | 11.1                                 | 155                                             | 14                   |

Table 3. Bioconcentration of pesticides by aquatic invertebrates for model II (primary food organisms allowed to pick up pesticide from glass surface and then given to the test organisms).

| Test organism (primary food) | Pesticide | Original amount, μg (theoretical concentration, ppb) | Final concentration found, ppb | Concentration factor |
|-----------------------------|-----------|-------------------------------------------------------|---------------------------------|----------------------|
|                             |           | Water aquarium                                       | Test organisms                  |                      |
| Daphnia (algae)             | Dioxin    | 6.48 (162)                                           | 0.4                             | 879                  | 2,198                |
|                             | DDT       | 3.58 (179)                                           | 22.9                            | 43,123               | 1,883                |
|                             | Zectran   | 2.22 (111)                                           | 15.1                            | 37,499               | 2,483                |
| Ostracod (algae)            | Dioxin    | 6.48 (162)                                           | 2.6                             | 279                  | 107                  |
|                             | DDT       | 3.58 (179)                                           | 50.8                            | 36,391               | 716                  |
|                             | Zectran   | 2.22 (111)                                           | 43.5                            | 6,177                | 142                  |

* Measured against the final pesticide concentrations actually found at the end of the test.
Table 4. Bioconcentration of pesticides by aquatic organisms for model III (pesticides introduced into system in the form of residues on sand).

| Test organism | Pesticide | Amount of pesticide, µg | Concentration found, ppb Water (including food) | Test organisms | Concentration factor |
|---------------|-----------|--------------------------|-----------------------------------------------|----------------|---------------------|
| Brine shrimp  | Dioxin    | 1.62                     | 0.1                                           | 157            | 1,570               |
|               | DDT       | 1.79                     | 0.5                                           | 3,092          | 6,184               |
|               | γ-BHC     | 1.47                     | 5.2                                           | 495            | 95                  |
|               | Zectran   | 1.11                     | 5.0                                           | 89             | 18                  |
| Mosquito larvae | Dioxin   | 1.62                     | 0.45                                          | 4,150          | 9,222               |
|               |           | 3.24                     | 2.40                                          | 12,000         | 5,000               |
|               | DDT       | 1.79                     | 0.85                                          | 14,250         | 16,765              |
|               |           | 3.58                     | 1.40                                          | 30,200         | 21,571              |
|               | γ-BHC     | 1.47                     | 6.6                                           | 1,450          | 220                 |
|               |           | 2.94                     | 13.1                                          | 2,900          | 221                 |
|               | Zectran   | 1.11                     | 5.45                                          | 0              | 0                   |
|               |           | 2.22                     | 10.8                                          | 89             | 8                   |
| Fish (silverside) | Dioxin  | 1.62                     | 0.0                                           | 2              | —                   |
|               | DDT       | 1.79                     | 2.1                                           | 458            | 218                 |
|               | γ-BHC     | 1.47                     | 1.8                                           | 2,904          | 1,613               |
|               | Zectran   | 1.11                     | 4.7                                           | 213            | 45                  |

Table 5. Two-step bioconcentration of pesticide by mosquito larvae, and northern brook silverside (model III).

| Pesticide | Amount of pesticide, µg | Concentration found, ppb Water (including food) | Mosquito larvae | Fish |
|-----------|--------------------------|-----------------------------------------------|-----------------|------|
| Dioxin    | 1.62                     | 1.3                                           | 3,700           | 708  | 2,846 | 54  |
| DDT       | 1.79                     | 1.1                                           | 17,900          | 337  | 16,273| 306 |
| γ-BHC     | 1.47                     | 1.8                                           | 690             | 1080 | 383  | 600 |
| Zectran   | 1.11                     | 5                                             | 0               | 76   | 0    | 15  |

Table 6. Physicochemical characteristics of dioxin in comparison with other insecticides.

|          | Water solubility | Solvent solubility Water solubility | Partition coefficient (vs. hexane) | Benzene solubility, g/100 g |
|----------|-----------------|------------------------------------|-----------------------------------|-----------------------------|
| Dioxin   | 0.2 ppb         | $10^4$                             | $1,000^*$                         | 0.047                       |
| DDT      | 1.2 ppb         | $10^6$                             | 100,000                           | 80                          |
| Zectran  | $>100$ ppm      | $10^4$                             | 100 $^*$                          | —                           |
| γ-BHC    | 10 ppm          | $10^4$                             | 1,700 $^*$                        | 80                          |

* Estimates.

suspected. For instance, the pattern of bioaccumulation and concentration in fish is quite different from those in other organisms studied, in that both γ-BHC and zectran show higher degrees of affinity than DDT and TCDD, respectively. Although the data are not sufficient to permit a definite conclusion, they suggest the possibility that water-soluble pesticides tend to accumulate in fish.
The data indicate that TCDD is not likely to accumulate in as many biological systems as DDT. This is likely because of TCDD's low solubility in water and lipids as well as its low partition coefficient in lipids. Since microbial degradation is not expected to be a major factor, the predominant mode of elimination of this compound in the environment is photodecomposition by sunlight, (9).

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