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Exposure of Forest Herbivores to 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in Areas Sprayed with 2, 4, 5-T*

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INTRODUCTION

The productivity of forest resources is being enhanced by major reforestation programs in several regions of the United States. These programs emphasize the rehabilitation of forest lands that have become so dominated by noncommercial species that regeneration of crop species has become impossible. Herbicides, particularly 2,4,5-T and silvex, play a prominent role in reforesting such brushfields. In fact, 2,4,5-T may be the pesticide used most in forests of the United States, an estimated total of slightly more than a million pounds yearly.

Both 2,4,5-T and silvex are manufactured from trichlorophenol, which contains trace amounts of 2,3,7,8-tetrachlorodibenzodioxin, known as dioxin or TCDD. This contaminant forms when trichlorophenol is produced from tetrachlorobenzene. Under present production technology, the concentration of the TCDD in 2,4,5-T and silvex is minute, averaging 0.02 parts per million (ppm) or less in the 2,4,5-T acid (POSS[1], ERNE[2], NEW ZEALAND DEPARTMENT OF HEALTH 1977).

Much controversy about the continued use of 2,4,5-T and silvex in forestry and elsewhere has focused on TCDD--whether TCDD causes cancer or birth defects and whether it accumulates in ecosystems to harmful levels in the human food chain. This paper consolidates evidence that clarifies the likelihood of finding TCDD accumulations in ecosystems and of overlooking accumulations potentially harmful to humans or wildlife.

The controversy has been heightened by the extreme toxicity of TCDD and allegations of toxicity at levels only marginally higher than the lowest levels detectable. Therefore, some system for improving the sensitivity of detection is desirable to ensure that small but potentially harmful quantities are detected, if present.

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[1]POSS, R., unpublished paper presented at the Herbicide Seminar Western Environmental Trades Association, Portland, Oregon (1977).

[2]ERNE, K., Swedish National Veterinary Institute, Stockholm, Sweden. Personal communication, May 29, 1977.

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KOCIBA et al. (1978) recently completed a study significant in several respects--two are important here. First, by feeding one mammalian species (Sprague-Dawley rats) a diet containing 22 to 210 parts per trillion (ppt) and producing no irreversible toxic effects during a lifetime of exposure, they defined a TCDD level from which a safety factor could be established. Once a reasonable safety factor below the "no effect" level is accepted, a tolerance can be established for TCDD in the forest food chain. Second, the study established that the livers of rodents continuously fed sublethal TCDD-spiked diets consistently accumulated TCDD residues nearly 25 times the concentration in the diet (Table 1).

TABLE 1

TCDD Concentrations in Diet, Liver, and Fat of Sprague-Dawley Rats after Lifetime Feeding Tests.[a]

	TCDD (ppt)					
	Liver				Ratio of TCDD levels	
Diet	Upper	Lower	Mean	Fat	Liver/Diet	Fat/Diet
2,200[b]	28,000	20,000	24,000	8,100	10.9	3.7
210	5,800	4,600	5,100	1,700	24.3	8.1
22	600	500	540	540	24.5	24.5

[a]Source: KOCIBA et al. (1978).

[b]This level produced some mortality attributable to acute effects.

From the above work, a safety factor of 100:1 suggests that a lifetime chronic ingestion of 0.22 to 2.1 ppt would be tolerated without adverse effects by a much larger array of mammals. The safety factor represented by such concentrations would provide a substantial margin below the lowest level producing irreversible intoxication, such as tumor formation. The same concentration in a short-term exposure would have a much larger safety factor.

Unfortunately, TCDD concentrations below 10 ppt cannot be quantified, so the establishment of such tolerances has not proven useful. However, in the data of KOCIBA et al. (1978), we observe a consistent direct relationship between TCDD concentrations in the diets and livers of reasonably healthy animals. Because the relation works both ways, it should be useful for determining low concentrations in diets by dividing the observed liver concentration by the liver/diet ratio (Table 1).

A conventional application of 2,4,5-T (2.2 kg/ha) containing 0.02 ppm releases 44 µg TCDD per hectare, of which some portion presumably reaches foods consumed by forest mammals. Maximum

vegetation contamination with normal use of 2,4,5-T yields a residue of 11 to 115 ppm of 2,4,5-T (NORRIS et al. 1977) containing 0.22 to 2.3 ppt of TCDD at the instant of application.

Three studies suggest that such concentrations do not enter the food chain; to our knowledge, no data to the contrary have been confirmed. The most direct evidence is detailed here for the first time; the other two studies substantiate the apparent form of the direct evidence.

The direct evidence comes from a study of the ecological effects of phenoxy herbicides on western Oregon brushfields (NEWTON 1975). As part of that study, 2.2 kg/ha of 2,4-D and 2.2 kg/ha of 2,4,5-T (as the butyl ester in 143 l diesel fuel) were aerially sprayed on 40 hectares in mid-May when foliage was just emerging from buds and the spray's penetration through the forest canopy was near maximum.

Feeding and burrowing activities of various mammals were recorded at 100 permanent vegetation sampling stations. In addition, mountain beaver (Aplodontia rufa) were live-trapped and examined for pathological evidence.

Mountain beaver are useful for determining environmental exposure to contaminated food in a forest. Their home ranges are small, and they live underground where they store foraged fern fronds and other herbs from the forest understory (HOOVEN 1977). Stored out of sunlight which can quickly degrade TCDD, this vegetation might be expected to retain maximum levels of TCDD in the diet of this mammal. In western Oregon, their principal foods are sword fern (Polystichum munitum), vine maple (Acer circinatum), and salmonberry (Rubus spectabilis). Each effectively intercepts light or spray droplets. All were in partial or full foliage during the herbicide application, while red alder (Alnus rubra) overstory was still nearly open. The pattern of observed damage to alder, salmonberry, vine maple, and other woody species testified to the targeting of the expected deposit of herbicide on the trapping site.

Ten "treated" mountain beavers were captured during a 2-week interval beginning 45 days after herbicide application, and one "control" animal was captured outside the treated area. All exposed animals had home ranges centered at least 300 feet inside the treated area, ensuring that their entire food supplies had been exposed to the herbicide; the control animal was caught more than a mile from the edge of the plot. Five other "control" animals were captured in 1978 to broaden the base for observations of animals with no exposure to phenoxy herbicides.

During the trapping periods, the wire cage-traps were checked each morning to insure minimal trauma to the captured animals. Captured animals were transported in the cage-traps to the Veterinary

Diagnostic Laboratory at Oregon State University. Each animal was euthanized and immediately received a complete histopathological examination, with special attention to the liver and lymphoid tissue.[3] All animals except one were euthanized by intercardiac injection of barbiturates. One control was chloroformed to insure that none of the observed microanatomical features were biased by the euthanasia.

The livers of all animals were grossly very black and showed an indistinct lobular pattern. The black pigmentation of the liver was the same whether the animals were euthanized by barbiturates or chloroform. Four "treated" animals had moderate body fat; the six other "treated" animals and the "controls" had negligible adipose tissue. One "treated" animal and several "controls" had numerous pinpoint black foci scattered over the parietal surface of both lungs.

The livers and other selected parenchymatous organs of each mountain beaver were microscopically examined. All "treated" animals had histopathologically normal livers. In the livers of both treated and untreated animals, hepatocytes, especially those nearest the portal triads, had a peculiar granular pigmentation. Special staining characteristics suggested that this pigment was melanin, apparently a normal characteristic of the species. (SNYDER and NEWTON 1975).

Hepatocytes in the liver of the 1973 "control" animal varied remarkably in size. Giant liver cells with huge hyperchromatic nuclei, suggesting polyploidy, were scattered throughout the liver without any distinct relationship to the liver lobules. This hepatomegalocytosis is seen commonly in other animals with mild exposure to intoxicating substances such as aflatoxins, alkaloids, or polychorinated biphenyls. Its occurrence only in the control animal cannot be explained unless the animal had been feeding on the noxious plant, tansy ragwort (Senecio jacobea). Tansy was abundant outside the treated area, but it had been temporarily controlled in the treated area by the herbicide.

The animals appeared in good physical condition and had none of the gross or microscopic lesions symptomatic of dioxins in liver or lymphoid tissue.

As each animal was examined, its liver was carefully removed and prepared for TCDD analysis. To avoid exposure to plastic or other contaminating surfaces that would interfere with TCDD analysis, each liver was separately wrapped in aluminum foil,

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[3]Appreciation is expressed to Dr. Jack Schmitz, of the Oregon State University (OSU) Veterinary Diagnostic Laboratory for his services in the examination of the 1978 specimens, and to Catherine Roberts and Dr. E. F. Hooven of the Forest Research Laboratory for helping capture the animals.

labeled, and placed in a glass jar sealed with aluminum foil. The livers were immediately frozen and held at -20°C until shipment in dry ice to the Dow Analytical Services Laboratory in Midland, Michigan[4]. Ten-gram samples were prepared (SHADOFF et al. 1977) for analysis with a LKB-9000 gas chromatograph-mass spectrometer set to monitor mass equivalents (m/e) of 320 and 322 for detecting TCDD containing random inclusions of Cl_{37}, as well as the more common Cl_{35}. Previous tests on biological samples showed that recovery (the ratio of observed to actual content) ranged from 68 to 120 percent, averaging between 85 and 90 percent.

The minimum detectable levels for the livers ranged from 3 to 17 ppt (Table 2). At this level, all samples were negative except for one "maybe." This sample had a component that showed a signal within 1 percent of the TCDD retention time and produced ions of m/e 320 and 322 in the approximate ratio for TCDD. That peak would calculate as a TCDD concentration of no greater than 3 ppt. The small size of this particular liver (5.5 g) precluded a duplicate analysis. Regardless of the actual composition of the detected material, it was at the lower limit of detection. The 1978 "control" livers were not analyzed for TCDD.

TABLE 2

TCDD Analyses in Livers of Mountain Beavers After Feeding for 45 to 60 Days in a Forest Sprayed with 2,4,5-T (2.2 kg/ha).

Sample	TCDD (ppt)	Analytical Detection Limit (ppt)
2050-73-1	ND[a]	11
2063-73-2	ND	13
2062-73-3	ND	17
2062-73-4	3(?)	3
2062-73-5	ND	5
2062-73-6	ND	9
2071-73-7	ND	3
2071-73-8	ND	10
2071-73-9	ND	9
2095-73-10	ND	8
2110-73-4 (Control)	ND	11

[a]None detected.

[4]Appreciation is kindly expressed to Capt. Alvin L. Young, U.S. Air Force Academy, who arranged for the financing of these analyses.

These are the only known livers systematically collected as TCDD indicators in the wildlife food chain. The generally negative findings suggest extremely low or negligible exposure. If 25:1 is accepted as the liver-to-diet ratio of TCDD concentration, these data show that the food supply for terrestrial mammals contains less than 0.125 ppt. This level is substantially less than 1 percent of the upper doses reported in the scientific literature as producing no effects in lifetime feeding studies (KOCIBA et al. 1978).

These data are supported elsewhere. The Dioxin Monitoring Programs[5] reported finding no quantifiable TCDD in the livers of livestock on treated rangelands. Three of 83 beef cattle showed detectable TCDD (20-60 ppt) in their fat, but the same animals surprisingly had no TCDD accumulations in their livers. This is remarkable considering that rats consistently had higher concentrations in liver than in fat (KOCIBA et al. 1978). It also may reflect the great difficulty in analyzing for TCDD in samples where other fat-soluble compounds can cause interfering analytical signals.

We assume the mountain beaver had no choice but to feed on contaminated food. The sword fern, in particular, is its staple food, and the orientation and phenology of sword fern would lead to its maximum contamination with the late dormant spray used. Consequently, the mountain beaver is a "worst case" example.

NEWTON and NORRIS (1968) observed that deer killed in areas treated with 2,4,5-T or atrazine had consumed very low levels of herbicides. Herbicide concentrations in the rumen during the first 6 weeks after treatment ranged from 0 to 1.31 ppm for each kilogram of active ingredient applied per hectare. The deer feeding in areas treated with 2,4,5-T had lower herbicide levels in their rumens than did those feeding on atrazine-treated areas, suggesting some selectivity in foraging (Table 3).

Assuming that vegetation is contaminated with 1 ppm of herbicide, three times the highest level observed in the stomach contents of deer feeding on phenoxy-treated forage, an animal feeding on foliage newly treated with 2,4,5-T would be consuming 0.02 ppt of TCDD. For chronic exposure to occur, TCDD degradation would have to be negligible over an extended period. This seems unlikely considering that TCDD in sunlight degrades rapidly in the presence of a hydrogen donor such as oil or 2,4,5-T (CROSBY and WONG 1977). The treatment regime that we used met all the apparent requirements for such degradation, with the possibility that the rate may have been reduced by partial shade and cool conditions. Therefore, deer and other forest herbivores seem unlikely to accumulate TCDD to detectable levels.

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[5]U.S. ENVIRONMENTAL PROTECTION AGENCY: Dioxin position statement. Prepared by the Dioxin Working Group. Washington, D.C. (April 28, 1977).

TABLE 3

Herbicide Concentrations in Rumens of Blacktail Deer After Feeding in Treated Areas.[a]

Herbicide Applied (kg/ha)	Days Between Treatment and Kill	Herbicide Detected and Concentration in Rumens (ppm)	Concentration in Rumen per Rate of Herbicide Application (ppm/kg/ha)
None	--	2,4-D (0.024)	b
		2,4,5-T (0.022)	b
Atrazine (4.4)	10	atrazine (5.76)	1.31
2,4,5-T (2.2)	15	2,4,5-T (<0.006)	<0.003
Atrazine (4.4)	17	atrazine (3.45)	0.78
Atrazine (4.4)	26	atrazine (0.44)	0.10
2,4,5-T (2.2)	31	2,4,5-T (<0.006)	<0.002
2,4,5-T (2.2)	43	2,4,5-T (0.358)	0.16
		2,4-D (0.088)	b
Atrazine (4.4)	44	atrazine (0.428)	0.10

[a]Source: Adapted from NEWTON and NORRIS (1968).
[b]We have no evidence that these animals were exposed to herbicides not included in the treatment or applied to nearby areas; therefore, we cannot explain these observations at close to the detection limit.

Our findings of negative or negligible contamination are consistent with the reports by SHADOFF et al. (1977) and the U.S. Environmental Protection Agency. They provide reassuring evidence that TCDD contamination does not pose a threat to forage and wildlife. Furthermore, the liver's affinity for TCDD suggests that TCDD can be detected long before it accumulates to harmful levels. The low levels in wildlife directly exposed in treated forests also suggest that a source other than forest applications should be suspected if TCDD appears in the tissues of humans without direct and prolonged exposure.

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