

Research Communications

Enhanced Co-Metabolism of TCDD in the Presence of High Concentrations of Phenoxy Herbicides

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Abstract. Chemical residue studies were conducted from 1977–1987 on sites where spills of Agent Orange had occurred in the Herbicide Storage Sites at the Naval Construction Battalion Center, Gulfport, Mississippi, and on Johnston Island, Central Pacific Ocean. The soil persistence time of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was significantly decreased when in the presence of massive amounts of phenoxy herbicides (> 62,000 µg of herbicide/g of soil). Although microbial populations doubled in the most highly contaminated sites, fungal species diversity decreased. The dominant fungal species that appeared to be associated with the metabolism of the residues were of the genera *Penicillium*, *Mucor*, and *Fusarium*. TCDD level decreased from a mean high of 180 ng/g (ppb) to less than 1 ng/g of soil over a ten-year period.

Introduction. The herbicide 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) was widely used as a commercial brush killer, and was a major component of the defoliant 'Agent Orange' in Vietnam [1]. The use of the herbicide was terminated when it was found to contain the teratogenic contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [2]. The environmental conditions influencing the fate and persistence of TCDD in the soil have been thoroughly studied [3]. For example, when present in the *n*-butyl formulation of 2,4,5-T (as in Agent Orange) and aerially applied, the TCDD was readily susceptible to photodegradation. Thus, the TCDD in Agent Orange was shown to photodegrade while dispersed in the air, and on soil and vegetative surfaces [3]. However, repetitive applications over a short-time span, such as occurred at Eglin Air Force Base, Florida, in the development and testing of the aerial spray equipment for Vietnam, the TCDD was found to accumulate and persist at a mean level of 0.3 ng TCDD/g of soil (ppb) for more than 14 years after application within the top few cm of the soil surface [4]. But what happens to the TCDD when large quantities of 2,4,5-T (e.g., in Agent Orange) are spilled, as occurred in the former Herbicide Storage Sites in both Vietnam and in the United States during and after the Vietnam War [5]?

Method. To determine soil persistence of both the herbicide and TCDD, two former Agent Orange Storage Sites were studied. In April 1970, at the termination of the use of Agent Orange in Vietnam, the United States Air Force re-drummed

all remaining stocks of the herbicide in Vietnam (5.24 million L) and shipped it to Johnston Island, Central Pacific Ocean, where it was placed in open storage from April 1972–July 1977. Simultaneously, the remaining stocks of Agent Orange (3.3 million L) that were not shipped to Vietnam were placed in open storage at the Naval Construction Battalion Center (NCBC), Gulfport; Mississippi, from December 1969 to June 1977. Despite constant efforts to redrum and maintain the integrity of the inventories, significant quantities of herbicide leaked or were spilled at both storage areas (> 80,000 L at Johnston Island). In 1977, both inventories were destroyed by at-sea incineration, and a site program was initiated to monitor for residual herbicides [2,4,5-T, and 2,4-dichlorophenoxy acetic acid (2,4-D), the other herbicide in Agent Orange] and TCDD.

The protocol developed for the selection of sites where soil samples were to be collected within the two 5-ha storage areas was based upon the determination of whether a spill had occurred, and whether the herbicide 'stain' was discernible as heavy, light, or absent. Because the herbicide also had a strong and distinct smell, the use of herbicide odor was used as a measure to also identify the age of the spill, i.e., whether the odor was detectable as strong, mild, or absent. Thus, within each storage area numerous locations were found that had a heavy stain and strong odor (labelled H/H, presumably representing a recent spill); a light stain and mild odor (labelled L/L, presumably representing an old spill); and, no stain and/or odor (O/O, presumably representing an uncontaminated area. Fourteen replications of each treatment were then randomly selected to represent the storage area, thus a total of 42 permanently marked sampling locations at both NCBC and Johnston Island. At both locations maps were constructed with the sampling points identified. Forty-two samples were taken in January 1978, and periodically over the next four years. In collecting the samples, an 8-cm square was marked 15 cm from the site marker pin. At each sampling date, an 8-cm cube of soil was taken from a different 'point-of-the-compass' with reference to the marker pin to ensure a 'fresh' and undisturbed profile. Each sample was analyzed for 2,4-D, 2,4,5-T, dichloro- and trichlorophenols, and TCDD; and, in January 1978 all samples were additionally subjected to a microbial analysis. Composite soil samples from the HH sites were taken in 1987.

Table 1: Mean concentrations, $\mu\text{g/g}$ of soil, of total herbicides, total phenols, and TCDD in the top 8 cm of soil samples collected in January and November 1978 from the former Agent Orange Storage Site at NCBC, Gulfport, Mississippi

Location	Number of Sites (Samples)	Total Herbicides (ppm)	Total Phenols (ppm)	TCDD (ppm)
'No' Spills (O/O)				
January 1978	14	32 α	3.5 α^a	ND (4) ^b
November 1978	14	3 β	0.4 β	NA
'Old' Spills (L/L)				
January 1978	14	1,202 α	86 α	0.0364 (3)
November 1978	14	492 β	23 β	0.0438 (3)
'New' Spills (H/H)				
January 1978	14	51,285 α	437 α	0.2064 (10) α
November 1978	14	30,005 β	253 β	0.1444 (11) α

^a Using the Wilcoxon Paired-Sample Test, means within columns within subtitles followed by the same letters are not significantly different at the 0.05 probability level

^b ND = Not Detected; NA = Not Analyzed, () number within the parentheses refers to number of positive samples used in calculations of the means

Results. A sample summary of the analytical results for the 42 sites sampled in January and November 1978 at NCBC is shown in Table 1. As noted in the table the protocol of using 'sight and smell' was effective and consistent for determining both the presence and age of a spill. The significant differences in both the herbicide and TCDD levels between old and new spills suggested that factors other than photodegradation were responsible for the rapid disappearance of chemical residues. Additional studies confirmed that in H/H sampling locations, 95% of the herbicide and TCDD residues were located within the top 8 cm of soil surface, but as the spill site aged, 50% of the remaining herbicide was found in the 8–16 cm depth of the soil profile. However, greater than 85% of the remaining TCDD was consistently found in the top 8 cm of the surface regardless of age of the spill. Similar data were found for samples from Johnston Island. At NCBC studies of soil microbial populations collected in January 1978 found that recent spills of herbicide contained almost twice the number of bacterial organisms per gram of soil, but a significant decrease in diversity of fungal species. Only two fungal species were found in H/H sites (*Penicillium* spp. and *Mucor* spp.), while L/L sites contained six different fungal species. The 'No Spill' sites (O/O) and a control site away from the former storage areas, contained 13 identifiable fungal species. For the Johnston Island Herbicide Storage Site, 13 fungal genera were also identified within the compacted coral that under-laid the storage site. As with NCBC, two fungal genera dominated the H/H sites, including *Fusarium* sp. and *Penicillium* spp.

Discussion. Studies of the soil residues of 2,4-D and 2,4,5-T confirmed the rapid disappearance (degradation) of the herbicides in the first year of monitoring (see Table 1). Significant differences in both herbicide and TCDD levels were noted between new and old spills suggesting that soil microflora may have played a role in the degradation of both the herbicide and TCDD. Indeed, the higher the initial residue levels, the greater the impact on the fungal populations. In soils with the highest levels of residue, large numbers of *Penicillium* spp. and *Mucor* spp. dominated the sites and likely played an important role in co-metabolism. Although data not shown, in both locations at NCBC and Johnston Island, the level of total herbicides decreased from a maximum of 62,000 $\mu\text{g/g}$ (ppm) to less than 2% of the initial concentration remaining at the

end of 4 years. In the same sampling period and sample sites, the mean TCDD concentration decreased from 180 ng/g (ppb) to less than 100 ng/g. In both storage sites sampled in 1987 (10 years after removal of drums), levels of TCDD in composited H/H soils samples ranged from 0.6 ng/g (ppb) to 1.0 ng/g (ppb). The loss of herbicides and TCDD was attributed primarily to microbial degradation, with some likely volatilization with subsequent photodegradation.

Conclusion. Old and new spills of Agent Orange in storage sites at the Naval Construction Battalion Center (NCBC) and Johnston Island differed significantly in the levels of 2,4-D, 2,4,5-T and 2,3,7,8-TCDD residues. Although spills may have occurred over a five- to seven-year period, respectively, results suggested that a loss of approximately 98% of the herbicides and 83% of the TCDD occurred between spills that were identified as 'old' or 'new'. Studies of microorganisms in the top 8 cm increment of soil or coral of NCBC and Johnston Island, respectively, confirmed a close relationship between high concentrations of herbicides and TCDD, and high population numbers of bacterial and fungal organisms. The most striking observation at both storage sites was that organism found proliferating in the soil or coral with high concentrations of herbicides and TCDD were essentially not found in other sites.

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