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Corporate Author

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LABORATORY INVESTIGATION FOR MICROBIOLOGICAL DEGRADATION
OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN SOIL ADDED OF
ORGANIC COMPOST.

(I. Camoni, A. Di Muccio, D. Pontecorvo and L. Vergori.
Istituto Superiore di Sanità, Viale Regina Elena, 299 - Roma)

An experimental investigation having the aim of studying and/or assessing the effectiveness of proposed methods for microbiological degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in polluted soil of Seveso (Italy) has been carried out.

Microorganisms' activity on the degradation process of TCDD in soils has been investigated by means of two different laboratory experiments in which an organic compost was used to provide a large number of microorganisms.

The experiments were developed along the following lines:

- i) Samples of soil taken in the "Zone A" of Seveso (Italy) containing an average of 0.1 mg/Kg TCDD were analyzed in absence and in presence of the compost;
- ii) Samples of TCDD free soil, to which TCDD was added at level of 0.1 mg/Kg, were also analyzed in absence and in presence of the compost. In these samples also peptone was added.

During the experiment, all the samples, contained in beakers, were stored in a non temperature-conditioned room (temperature between +5°C and +28°C) and were protected from direct sunlight. At regular intervals, 25 ml of distilled water were added to each sample to keep the soil moistened. Samples were analyzed over a period of 480 days at increasing intervals.

Under the adopted conditions, both the experiments showed a reduction of TCDD content of about 25% in 480 days.

Statistical evaluations of the experimental data emphasize that the observed decay is significant, but it is not related to the addition of the compost and peptone, which does not seem to exert any appreciable influence.

Chemical analyses of TCDD were carried out by gaschromatography-mass fragmentography using our previously published method.

S. CERQUIGLINI MONTERIOLO, A. DI DOMENICO, V. SILANO, G. VIVIANO,
G. ZAPPONI, ISTITUTO SUPERIORE DI SANITA', ROME, ITALY

2,3,7,8-TCDD LEVELS AND DISTRIBUTION IN THE ENVIRONMENT AT SEVESO
AFTER THE ICMESA ACCIDENT ON JULY 10th, 1976

Environment contamination at Seveso after the industrial accident involving the emission of a TCDD-containing toxic cloud was assessed by analyzing representative samples of different matter in the environment (soil, water, silt, dust particles, plant and animal tissues). TCDD levels in the soil surface layer ranged from <0.75 to $\sim 20 \times 10^3 \mu\text{g}/\text{m}^2$. The territory involved was divided into three zones on the basis of soil contamination levels. TCDD levels were highest in Zone A whereas Zones B and R exhibited <50 and $<5 \mu\text{g}/\text{m}^2$ levels, respectively. Vertical distribution of TCDD in the soil has been studied. A sharp decrease in TCDD levels was observed as depths increased. TCDD was also monitored in suspended and settleable air particulates using high-volume samplers or dustfall jars. TCDD levels in these samples ranged from 0.06 to 2.14 ng/g of dust for dustfall jar specimens and from 0.17 to 0.050 ng/g of dust for suspended particulates. No TCDD was detected in drinking, surface, or ground water samples (method sensitivity: in the ppt range). Traces of TCDD in the ppt range were occasionally found in the silt analyzed at the end of 1976 and early 1977. Maps were given to show territorial distribution of TCDD over the area concerned.

STUDIES ON MICROBIAL METABOLISM OF TCDD UNDER LABORATORY CONDITIONS

Extensive measurements on the behaviour of TCDD in soil under natural conditions¹ or in the laboratory^{2,3} suggested a half-life of appr. 1 year. It was postulated, that soil microorganisms may contribute to the assumed decay. In aquatic ecosystems no metabolism of TCDD was observed or at least a half-life of 600 days⁵. Laboratory experiments gave indications, that some microorganisms might be able to attack TCDD to a limited extent⁶.

As a consequence of the Seveso accident (July 10, 1976) we initiated a programme to study under laboratory conditions the microbial metabolism of TCDD by pure or mixed cultures in liquid nutrient media and in nutrient enriched moist soils. The study was performed in closed systems at 28°C. 0,5 - 1 ppm of ¹⁴C-labelled TCDD (spec.act. appr. 100 mCi/ mmole) was added to the cultures. Analysis was performed with TLC plates and with a Radio-GC-System (Packard Instruments; GC model 427, Radiosystem 894); CO₂ was captured in 0,1 KOH.

No strong microbial metabolism of TCDD could be detected, even after prolonged incubation of up to 1 year in liquid media or in soil samples. The extractability of TCDD from the samples decreased often considerably with prolonged incubation, simulating a decay. This difficulty has also been observed by other investigators^{2,3,5}. Careful and repeated extractions of the samples with various solvents increased the recovery of TCDD. Furthermore selective extraction of impurities present in the ¹⁴C-TCDD samples used suggested sometimes a metabolism of TCDD; but complete balances excluded this possibility. Our present data do not exclude a very slow metabolism of TCDD in the range of 1-2 % within 1 year by some of our strains.

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SILVIA CERLESI
UFFICIO SPECIALE DELLA REGIONE LOMBARDIA-SEVESO (COMO)
GIACOMO BRESSI, ELIO CALLIGARICH
ISTITUTO NAZIONALE DI FISICA NUCLEARE, SEZ. DI PAVIA-PAVIA
FABIO BOSSI, SERGIO P. RATTI
ISTITUTO DI FISICA NUCLEARE DELL'UNIVERSITA' DI PAVIA

GEOMETRICAL DISTRIBUTION OF TCDD ON THE SURFACE LAYER AROUND
ICMESA. AN ANALYTICAL DESCRIPTION OF THE MAIN FEATURES AND
THE DIFFERENT APPROACHES IN THE DIFFERENT MAPPING PROCEDURES.

Abstract

A chemical plant failure 13 miles north of Milan (Italy) has spread an amount of 2, 3, 7, 8-Tetrachloro-di-benzo-p-dioxin (TCDD) on a large and heavily populated area.

The data accumulated in different instants have been analyzed to give an overall geometrical description of the contamination due to the accident.

The analysis of the data made available shortly after the accident, as a result of an approximately equal-distance grid, has allowed the determination of the line of maximum contamination and has provided a number of indications useful to program a 1979 campagne based on a different grid.

SILVIA CERLESTI

UFFICIO SPECIALE DELLA REGIONE LOMBARDIA-SEVESO (COMO)

GIACOMO BRESSI, ELIO CALLIGARICH

ISTITUTO NAZIONALE DI FISICA NUCLEARE, SEZ. DI PAVIA-PAVIA

FABIO BOSSI, SERGIO P. RATTI

ISTITUTO DI FISICA NUCLEARE DELL'UNIVERSITA' DI PAVIA

ANALYSIS OF THE TCDD-DISTRIBUTION AS A FUNCTION OF THE UNDERGROUND DEPTH FOR DATA TAKEN IN 1977 AND 1979 IN ZONE A AT SEVESO (ITALY).

Abstract

The TCDD distribution as a function of the depth below ground has been investigated on the data provided by two monitoring campaigns during 1977 and 1979 respectively.

In spite of non trivial fluctuations, all the data can be reduced to an unique analytical form describing the distributions up to the maximum depth at which measurements have been done (1.5 m) with very reasonable statistical confidence level ($\sim 90\%$).

The shape of the empirical distribution (a conical form) describing the data of the 1977 campaign remains the same also for the data of the 1979-campaign and may give an approximate indication of the TCDD penetration in the soil as a function of time.

TCDD-levels in soil and plant samples from the Seveso area

H.-K. Wipf, E. Homberger, N. Neuner, U.B. Ranalder,
W. Vetter and J.P. Vuilleumier

Givaudan Research Laboratories, CH-8600 Dübendorf, Switzerland

* * *

Soil and vegetation samples have been collected in the Seveso area from 1976 through 1979 and analyzed subsequently.

In 1976, immediately after the incident, TCDD-concentrations in plant material reached the order of 1 ppm in the zone of highest contamination. In the following years, when there was no direct contact of the newly grown vegetation with the accidentally released aerosol cloud, TCDD-levels in plants dropped by several orders of magnitude.

In 1977, even in the most contaminated part of Zone A with soil concentrations in the order of 10 ppb (ca. 1'000 $\mu\text{g}/\text{m}^2$) no traces of TCDD were found in the flesh of apples, pears and peaches nor in corn cobs and kernels (detection limit 1 ppt). Traces of TCDD were detected, however, in the skin of the fruit and in the sheaths of the corn cobs. This strongly suggests that the contamination arises from local dust and not from plant uptake. Our studies of TCDD-distribution in carrots grown in heavily contaminated soil confirm the absence of a significant uptake of TCDD by plants.

No measurable amounts of TCDD have been detected in any of the vegetation samples (even carrots) collected in the scarcely contaminated Zone R from 1977 through 1979. This is in accordance with the very low soil residues in this safety-zone, ranging in 1979 from not measurable (< 1 ppt) in the vast majority of the area to ca. 10 ppt in a few localized spots.

A. DI DOMENICO, G. VIVIANO, G. ZAPPONI, ISTITUTO SUPERIORE DI
SANITA', ROME, ITALY

ENVIRONMENTAL PERSISTENCE AND MOBILITY OF 2,3,7,8-TCDD AT
SEVESO

TCDD levels were determined at 44 soil sites in Zone A during three surveys carried out at different times (1, 5 and 17 months) after the ICMESA accident. The data obtained provide statistically significant ($p < 0.01$) evidence that the geometric mean of TCDD levels dropped to about one-half in the unworked soil of Zone A in the first 5 months after the accident. Following this period, no further decreases in TCDD levels were detected. Available data are consistent with the hypothesis that TCDD presence in the Seveso environment diminishes with time, and at a rate which also decreases with time. The same pattern seems to apply for TCDD soil penetration. From one calculation it appears that one month after the accident, TCDD half-life was about 1 year, whereas 17 months after the accident, it was estimated to be >10 years.

Tetrachlorodioxins (TCDD) in Great Lakes Herring Gull Eggs

An intensive assessment of TCDD in the Great Lakes ecosystem has been completed using pools of 10 randomly selected Herring Gull eggs collected in 1979 from each of 2 colonies on the 5 Great Lakes (see map). These are the 10 monitor colonies of the Wildlife Contaminants Surveillance program which have been utilized by the Canadian Wildlife Service and the International Joint Commission Great Lakes Water Quality Board to monitor trends in persistent organochlorine contaminants (e.g., PCB's, mirex, DDE) in the Great Lakes aquatic ecosystem. Additional pooled samples were analyzed from colonies on the Niagara River, chosen for its proximity to the Hooker Chemical dumping site at Bloody Run, and Fighting Island in the Detroit River, which exhibited the highest organochlorine residues of any Herring Gull sample collected in 1979. Eggs collected from Scotch Bonnet Island (central Lake Ontario) in 1973 were also analyzed as a retrospective search for TCDD in L. Ontario samples. Chicks which hatched from this colony exhibited chick edema disease symptomatic of contamination by several toxic organochlorines, including hexachlorobenzene and hexachlorodioxins.

No tetrachlorodioxin isomers have been detected at or above a concentration of 10 ng/kg (parts per trillion) in these samples. The Great Lakes aquatic ecosystem has been exposed to TCDD via the atmosphere and via direct discharge. There is, however, no evidence of bioaccumulation into Great Lakes Herring Gull eggs. Herring Gulls are at the top of the food chain and have been shown to accumulate extremely high levels in eggs (50 times those in fish) of other organochlorine contaminants such as PCBs and mirex. It follows that: (1) Herring Gulls do not bioaccumulate TCDD and/or (2) levels of TCDD in the aquatic food web (i.e., fish) are extremely low.

The first hypothesis is supported by the short half-life of TCDD in rats (12 and 15 days for males and females, respectively) relative to persistent PCB isomers (84 days for hexachlorobiphenyl). Chlorinated dibenzofurans are similar in structure and toxicity to dioxins. A CWS study showed that chlorinated dibenzofurans were eliminated much more rapidly than PCBs when fed to mallard ducks. The latter hypothesis is supported by the instability of TCDD to UV irradiation, the irreversible adsorption of TCDD to particulate matter in air, soil, water, and sediment, and the apparent inefficiency of bioaccumulation of TCDD from food by fish.

DOUGLAS J. HALLETT*, ROSS J. NORSTROM, Canadian Wildlife Service, National Wildlife Research Centre, Ottawa, Ontario, K1A 0E7, FRANCIS I. ONUSKA, and MICHAEL E. COMBA, Inland Waters Directorate, National Water Research Institute, Burlington, Ontario.