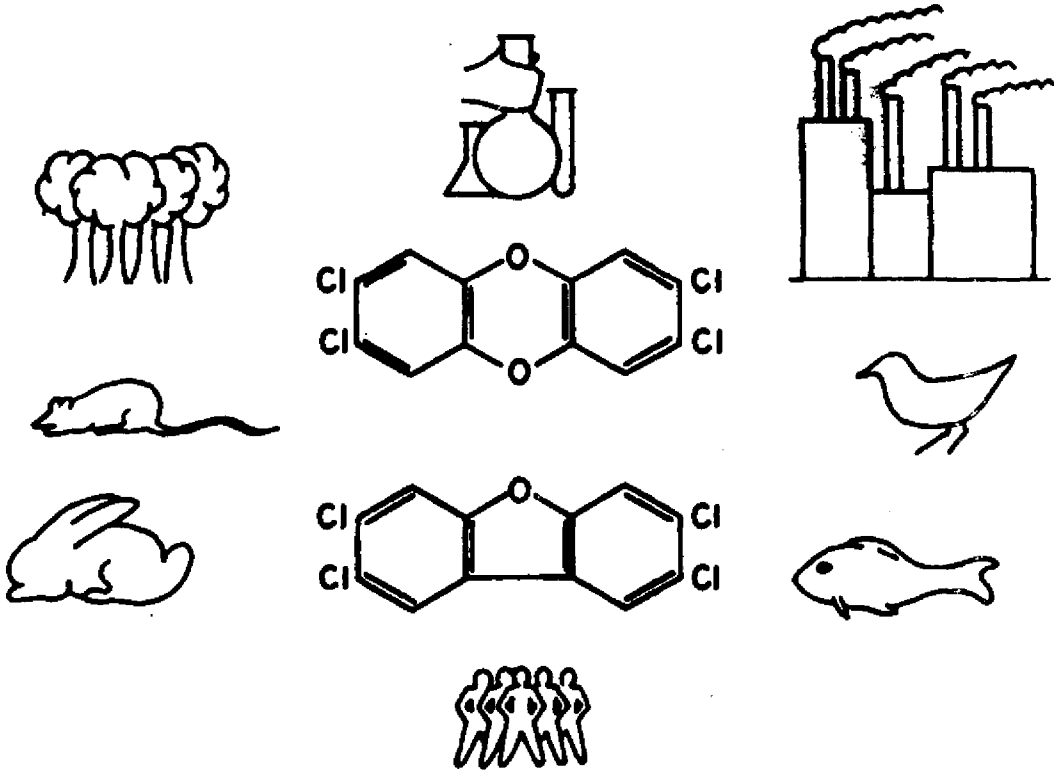
N.I.E.H.S. CONFERENCE

ON

CHLORINATED DIBENZODIOXINS

DIBENZOFURANS



SPONSORED BY

NATIONAL INSTITUTE

OF

ENVIRONMENTAL HEALTH

SCIENCES

Governors Inn

RESEARCH TRIANGLE PARK, N.C.

APRIL 2-3, 1973

MONS 206977

THE FORMATION OF DIBENZODIOXINS AND OTHER CONDENSATION PRODUCTS FROM CHLORINATED PHENOLS AND DERIVATIVES

H. G. Langer
Dow Chemical USA
Eastern Research Laboratory
Wayland, Massachusetts 01778

Conference on Chlorinated Dibenzodioxins and Dibenzofuran
National Institute of Environmental Health Sciences
April 2-3, 1973
Governors Inn, Research Triangle Park, North Carolina

Abstract

Chlorodioxins are formed in a two-step condensation reaction from ortho-substituted halophenoxy radicals or anions. Radical reactions are of significance only where strongly oxidizing conditions exist such as a reaction of chlorine with pentachlorophenol at elevated temperature. For anionic condensation we have investigated the strongly exothermic reactions of alkali metal salts of chlorinated phenols which occur at temperatures above 300°C. The product distribution depends on a variety of factors such as the total number of halogen substituents which determine the ease of removal of halogen, the arrangement of the molecules within a crystal lattice, if this reaction takes

MONS 206978

place in the solid state (which in turn is influenced by the metal cation involved), steric effects,which may facilitate the attack by the second molecule at a particular carbon or, on the other hand, may prevent a nucleophilic attack at a particular site, and finally an electronic effect,which in this case allows halogen atoms in meta position to be removed more readily than those in the other ring positions.

Only for sodium pentachlorophenate was the dioxin formation the major condensation reaction. Polychlorophenoxy phenols are the major reaction products for lower chlorinated phenols. Chlorinated dibenzofurans have generally only been observed in trace amounts.

The derivatives 2,4,-D, 2,4,5-T and silvex as well as their sodium salts and esters have not produced dioxins in pyrolytic reactions whether carried out in the solid state, in the melt,or in solution. Even after conditions of extreme hydrolysis,followed by pyrolysis we could observe only trace amounts of dioxins.

Even extreme conditions such as burning of treated wood or vegetation after the use of 2,4 -D, 2,4,5-T, silvex or their derivatives is not expected to produce detectable amounts of dioxins or dibenzofuran.

Analytical and Confirmative Techniques for. Dibenzo-p-dioxins Based Upon Their Cation Radicals

A. E. Pohland, G. C. Yang & N. Brown

Chlorinated dibenzo-p-dioxins form cation radicals when dissolved in strong acids in the presence of oxidizing agents or ultraviolet light. The presence and quantity of such cation radicals may be easily observed using electron spin resonance (ESR) as well as visible spectroscopic techniques. The applicability of these techniques to the quantitative estimation and confirmation of chlorinated dibenzo-p-dioxins in phenols and fats was therefore investigated.

A series of 13 chlorinated dibenzo-p-dioxins were prepared and optical and magnetic properties of the corresponding cation radicals were investigated. A wide variety of solvents of varying acid strengths were studied; it was established that the best solvent was trifluoromethane sulfonic acid. A series of oxidizing agents was also studied; the best of these for use in our optical studies was KNO_3.

In our studies of the visible spectra of cation radicals generated from chlorinated dibenzo-p-dioxins, we observed a general bathochromic shift with increased chlorine content. These shifts were also highly dependent upon the positions of the chlorine atoms, so that one could readily distinguish by the wavelength of the absorption maximum between the 1,2,3,4-, the 2,3,7,8- or the 1,3,6,8-tetrachloro isomers and between various other chlorinated dibenzo-p-dioxins. For the hexachloro and pentachloro isomers it became increasingly difficult to distinguish between isomers since all those isomers with chlorine atoms in the 2,3,7,8-positions absorbed maximally in the same region. With octachlorodibenzo-p-dioxin low solubility prevented immediate measurement; however, the compound did slowly dissolve thus offering a convenient time scale selectivity. A linear relationship between concentration and absorbance was observed for the 2,3,7,8-tetrachlorodibenzo-p-dioxin and the 2,7-dichlorodibenzo-p-dioxin over a concentration range of 0.4 to 3 µg /ml. The sensitivity of this method appears to be about 250 ng in a 1 cm cell for a signal to noise ratio of 5 to 1 using a Cary-14 spectrophotometer.

Large amounts of oxidizing agents were used in our ESR experiments in order to achieve maximum sensitivity. For convenience of measurement, the solutions were not degassed. Hence the hyperfine lines normally observed and characteristic of each chlorinated dibenzo-p-dioxin were not resolved. A linear relationship between concentration and intensity of the electron spin resonance signal was observed for several of the chlorinated dibenzo-p-dioxins including the 2,3,7,8-isomer for levels between 4 and 200 ng. The minimum detectable level of this isomer was found to depend upon the instrument sensitivity and the operation time constant, and is ca. 1 ng for a Varian E-9 spectrophotometer operating with a 3 second time constant and 40 mw input power.

The use of these techniques for quantitative estimation and confirmation of chlorinated dibenzo-p-dioxins will be discussed.

Determination of Chlorinated Dibenzodioxins and Dibenzofurans In Various Materials

W. B. Crummett and R. H. Stehl
Analytical Laboratories
The Dow Chemical Company

Methods used for the determination of chlorinated dibenzo-p-dioxins in various materials including chlorinated phenols, chlorinated phenoxy herbicides, ronnel, fat, and combustion products will be presented and critically examined. Special attention will be given to the validation of the methods and the certainty and uncertainty of the results. Data obtained over a 9-year period will be presented and its meaning explained. Analytical techniques investigated include gas chromatography, liquid chromatography, thin-layer chromatography, and gas chromatography-mass spectrometry. Chlorinated dibenzofurans can also be determined by the recommended procedure.

MDNS 206981

abstract of a report we would like to present under the subject heading

CHEMISTRY-ANALYTICAL METHODOLOGY

at the

NIEHS CONFERENCE ON CHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

April 1973

In view of the extraordinary toxicity of the chlorodioxins, unusually sensitive analytical methods are required to measure dioxins in environmental samples at levels of possible toxicological significance. Given that the single oral dose LD_{50} of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in guinea pigs is reported to be slightly less than one part per billion bodyweight, that in rats toxic effects are observed at about one one hundredth of the LD_{50}, and that a factor of about ten would be desirable for a safety margin, a desirable level of detection in animal tissue of $(10^{-9})(10^{-2})(10^{-1}) = 10^{-12}$ or one part per trillion bodyweight can be calculated. For a one gram sample the method used must have a sensitivity of about 10^{-12}g or one picogram. The method used should also be highly specific since at this level of sensitivity confirmation by other methods is difficult.

We describe a procedure involving extensive clean-up and mass spectroscopic analysis which meets those requirements. Approximately one picogram of TCDD can be detected with a high degree of specificity. The method separates TCDD from DDE, PCB's and other chlorinated hydrocarbon residues. The procedure has been demonstrated to be reproducible and to provide acceptable recoveries.

Robert W. Baughman
Department of Chemistry
Harvard University
Cambridge, Mass. 02138

Matthew S. Meselson
Department of Biochemistry
and Molecular Biology
Harvard University
Cambridge, Mass. 02138

MONS 206982

Various chlorinated dimers present in several technical chlorophenols, used as fungicides.

Sören Jensen and Lars Renberg

Special Analytical Laboratory

Swedish National Environment Protection Board, Stockholm, Sweden

February 1973

Dimers in pentachlorophenol[1]

The presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 2,4,5-trichlorophenoxy acid esters was shown to originate from the use of 2,4,5-trichlorophenol in the industrial process. The dimerization is thought to occur when the phenol is produced from the corresponding tetrachlorobenzene by the action of alkali.

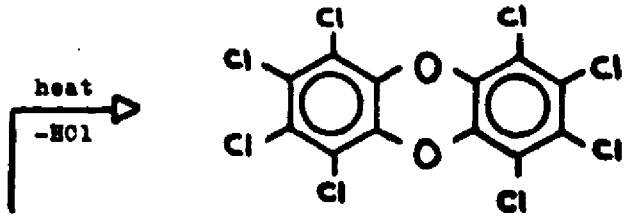
Logically all products originating from chlorinated benzenes treated with alkali are possible sources of chlorinated dibenzo-p-dioxins (chlorinated dioxins). We have therefore investigated the presence of chlorinated dioxins in some pentachlorophenol formulations.

The first result obtained was that the content of the expected octachlorodibenzo-p-dioxin in one formulation was 1000 ppm (1g/kg). However, the phenol-free extract was treated with diazomethane and reinjected into the gas chromatograph. Despite the fact that a dioxin cannot react with diazomethane, the octachloro-dioxin content decreased to 50 ppm. At the same time two new peaks appeared in the chromatogram.

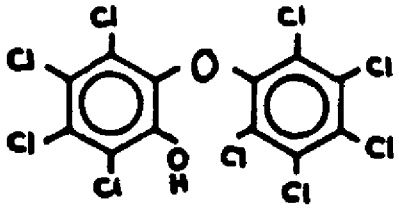
The compounds corresponding to these peaks were identified by means of mass spectrometry to be the methyl ethers of two isomeric nonachloro-hydroxydiphenyl ethers. The corresponding phenol to one of these ethers was later on proved to produce the octachloro-dioxin in the gas chromatographic column. Thus the first (high value) of octachloro-dioxin was the sum of the true dioxin and its precursor,

MONS 206983

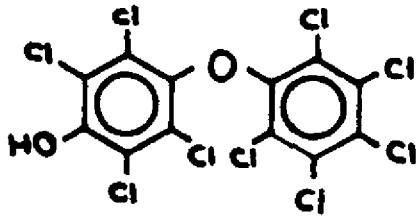
the hydroxydiphenyl ether. This compound was consequently given the name of predioxin. In the other diphenyl ether the hydroxy groups were not in a position allowing ring closure, and it was therefore given the name of iso-predioxin. The highest values found in the formulations investigated are given in the brackets in the figure.



octachloro-dioxin (D8)
(3300 ppm)



predioxin (PD8) (1600 ppm)



iso-predioxin (iso-PD8) (1600 ppm)

Besides these three substances, were also present an analogous serie originating from the reaction of one molecule of pentachlorophenol with one molecule of the impurity tetrachlorophenol. The corresponding values for the above mentioned formulation were D7 870 ppm, PD7 1300 ppm and iso-PD7 1200 ppm. The total content of these dimeric substances was thus 10 g/kg.

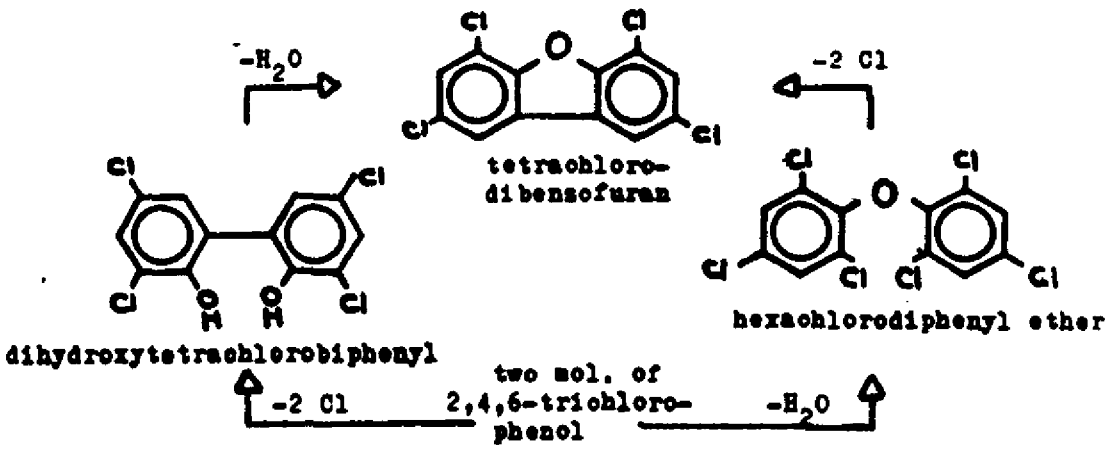
Dimers in 2,4,6-tri- and 2,3,4,6-tetrachlorophenols from direct chlorination of phenol[2].

Chlorinated phenols are also produced through chlorination of phenol by means of an iron-salt as catalyst.

No alkali is used in this process, but nevertheless dimers are formed. The amount is in some formulations up to 50g/kg.

In contrast to the products formed in the alkali process above, no dioxin was detected, but high amounts of both predioxins and iso-predioxins were found. The most probable predioxins expected are

the precursors to tetra- and hexachloro-dibenzo-p-dioxin respectively and the corresponding iso-predioxins.



Thus it seems that heat and/or alkali favour a ring closure resulting in the formation of a dioxin.

However, chlorinated dihydroxy biphenyls, not known to be found earlier have been detected in formulations, produced via direct chlorination. These substances are formed by dechlorination of two molecules of phenol, and may through dehydration produce chlorinated dibenzofurans. This reaction may be responsible for the presence for the dibenzofurans.

An alternative route may be dechlorination of the chlorinated diphenyl ethers, also found in these formulations.

References

1. Jansson, S. and Renberg, L. Contaminants in Pentachlorophenol: Chlorinated Dioxins and Predioxins (Chlorinated Hydroxy-Diphenyl ethers). Ambio Vol. 1, No.2. p 52-59
2. Nilsson, C.A. and Renberg, L. Further Studies on Impurities in Chlorophenols. To be published.

Technical Pentachlorophenol: The Origin and Analysis of Base-Insoluble Contaminants

Jack R. Plimmer
Pesticide Degradation Laboratory
Agricultural Research Service
United States Department of Agriculture
Beltsville, Maryland 20705

Abstract

Pentachlorophenol has been used as a herbicide, defoliant, insecticide and fungicide. Fats from hides preserved with technical pentachlorophenol and used as feed additives have been implicated as a source of chick edema factor. Chick edema disease was responsible for the death of millions of broiler chicks in 1957. Causative agents are chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans. Compounds of these two classes were detected in many samples of technical pentachlorophenol from commercial sources. Polychlorinated dioxins and furans and other polychlorinated aromatic compounds have been identified in the neutral fraction of pentachlorophenol by gas chromatography, mass spectrometry and the combination of these techniques. Formation of base-insoluble impurities may occur during manufacture by chlorination of lower phenols at elevated temperatures. Some of the base-insoluble compounds have also been obtained by the action of light on chlorophenols.

NONS 206986

Studies on the Formation Potential and Presence of Chlorinated Dibenzofurans in Chlorinated Biphenyls

R. L. Martin, M. L. Porter, and I. H. Pomerantz, Bureau of Foods, Food and Drug Administration, Washington, D. C. 20204

We have considered the possibility that chlorinated dibenzofurans may arise by photochemical alteration of chlorinated biphenyls under appropriate conditions. Several model chlorinated biphenyls, and dibenzofuran itself, were irradiated in solution in organic solvents with or without added water. Air was not excluded. Information on the products formed was obtained by GLC and by GLC/MS. These results will be discussed.

A sample of a Japanese-produced commercial chlorinated biphenyl has been examined for evidence of chlorinated dibenzofuran trace impurities. Our preliminary findings will be presented.

Pentachlorobiphenyl did not produce chlorinated furans under test conditions used. Methylation in acetone solution did occur.

Dichlorobiphenyl did not produce chlorinated furans.

Chlorinated dibenzofuran undergoes some destruction under photochemical conditions

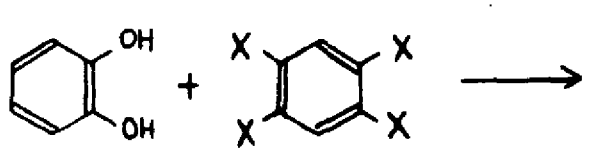
Kanechlor 400 (similar to Aroclor 1248) about 1 ppm tetrachlorodibenzofuran will confirm by MS. Preliminary indicates presence

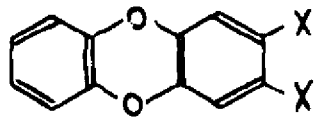
MONS 206987

SYNTHESIS OF NEW STERIC AND ELECTRONIC ANALOGS OF 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN. Andrew S. Kende and James J. Wade, Department of Chemistry, The University of Rochester, Rochester, N.Y. 14627.

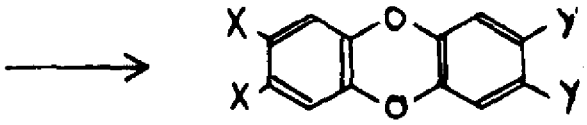
The availability of a rapid and reproducible enzymatic assay[1] which appears to parallel the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds has prompted us to initiate chemical studies to define the effects of structural parameters on biological activity. Of immediate interest was an attempt to distinguish steric from electronic requirements for toxicity in this series.

The intermediate 2,3-dichlorodibenzo-p-dioxin (I) is now available in good yield from the condensation of catechol with 1,2,4,5-tetrachlorobenzene by the method of Pohland and Yang.[2] We have observed that the corresponding tetrabromobenzene or tetrafluorobenzene reacts to yield the 2,3-dibromodibenzo-p-dioxin (II) or the 2,3-difluorodibenzo-p-dioxin (III). Each of these three intermediates undergoes clean chlorination under controlled conditions to produce TCDD, the new 2,3-dibromo-7,8-dichlorodibenzo-p-dioxin (IV), or the new 2,3-dichloro-7,8-difluorodibenzo-p-dioxin (V), respectively. Similarly, bromination of I, II or III yields IV, the 2,3,7,8-tetrabromodibenzo-p-dioxin (VI), or the 2,3-dibromo-7,8-difluorodibenzo-p-dioxin (VII).





I X=Cl, mp 159-160°
II X=Br, mp 155-156°
III X=F, mp 174-176°

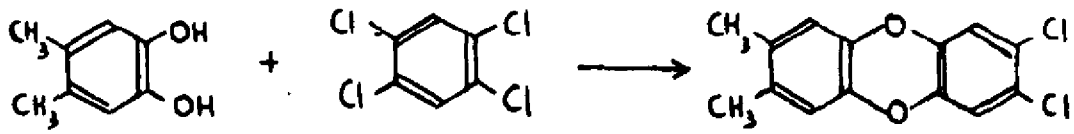


TCDD	X=Y=Cl
IV	X=Cl, Y=Br
V	X=Y=Br
VI	X=F, Y=Cl
VII	X=F, Y=Br

This route to TCDD, via chlorination of 2,3-dichlorodibenzo-p-dioxin, is particularly suitable for the microscale preparation of very pure uniformly labelled ^{14}C-TCDD of high specific activity.[3]

When 4,5-dimethylcatechol was employed in the above condensation the new TCDD analog VIII was obtained from 1,2,4,5-tetrachlorobenzene. Compound VIII is roughly isosteric with

TCDD but differs significantly in electron density of one of the rings and therefore in its overall polarity.



VIII

We are currently exploring the scope and limitations of the catechol condensation for a variety of polyhalobenzene electrophiles.

References

[1] A. Poland and E. Glover, Science, in press

[2] A. E. Pohland and G. C. Yang, J. Agr. Food Chem., 20, 1093 (1972)

[3] Cf. J. H. Vinopal, L. Yamamoto and J. E. Casida, Advan. Chem. Ser., in press; W. W. Mueller and L. A. Shadoff, ibid.,

Etiology of the Chick Edema Disease

David Firestone, Division of Chemistry and Physics, Office of Science, Bureau of Foods, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C. 20204.

(To be presentated at a NIEHS conference on Chlorinated Dibenzo-p-dioxins and Dibenzofurans, April 2 - 3, 1973 at the Governors Inn, Research Triangle Park, North Carolina)

Abstract

The chick edema disease was first encountered in the Unites States during the fall of 1957 when millions of broilers died in the eastern and midwestern parts of the country. Characteristic symptoms included the presence of excessive fluid in the heart sac and in the abdominal cavity accompanied by high mortality beginning in the third week. The cause was quickly traced to the presence in the feed of toxic by-products of the manufacture of commercial oleic and stearic acid. Even though the identity of the toxic factors was unknown, a suitable chick feeding bioassay and a gas chromatographic screening procedure were developed, and a food additive regulation was issued for fatty acids requiring that they be free of chick edema factors. Esrly work indicating that chick edema factors were chlorinated aromatic compounds was followed by later demonstration that they were a family of chlorodibenzo-p-dioxins. Subsequent investigation showed that (a) chlorophenols were precursors of the chlorodioxins, and (b) chlorodioxins and related compounds are commonly present as minor components in commercial chlorophenols. The chick edema disease outbreak in the late 1950's apparently arose from tallows and greases obtained as by-products from hides preserved with such chlorophenols prior to leather manufacture. A more recent outbreak (1969) of chick edema disease was traced to chlorophenol contamination of soapstock used as feed fats.

NONS 206990

UNIVERSITY OF CALIFORNIA
SAN FRANCISCO MEDICAL CENTER

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO
SANTA BARBARA • SANTA CRUZ

SCHOOL OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY

SAN FRANCISCO, CALIFORNIA 94122

A Survey of the Teratogenic Effects of 2,3,7,8 -Tetrachloro-dibenzo-dioxine in Mammalian Species.

Diether NEUBERT, P ZENS and A. ROTHENWALLNER

Dep.of Pharmacology(Sonderforschungsbereich Embryonal-Pharmakologie)
Free University Berlin, Germany

Although the toxic dose of 2,3,7,8.-tetrachloro-dibenzo-dioxine(TCDPD) for some mammalian species like rats and mice is comperatively high. clearcut teratogenic effects may be produced with TCDPD in these species with doses in the microgram-range.

Data on the teratogenic effects reported so fer in different species are summarised.

In our own studies speciel emphasis has been layed on dose-response relationships of the teratogenic effects. Furthermore, since the teratogenic effect of TCDBD is especielly interesting when the drug is given in combination with other teratogenic substances, series of experiments were performed to elucidate a possible potentiating effect of TCDPD.

Most of these experiments were performed in mice and cleft palate frequency was used as a criterion of the teratogenic effect

MONS 206991

POSTNATAL EFFECTS OF MATERNAL EXPOSURE TO TCDD

J. Moore, B. Gupta, J. Vos and J. Zinkl

Maternal exposure of C57Bl/6 mice to TCDD caused variations in fetal kidney maturation and development when compared to control. This response was dose related as scored by the number of fetuses affected and whether kidney involvement was unilateral or bilateral. In addition, the thymuses from treated litters were reduced in size although no effect on fetal weight was observed. To distinguish between effects due to a reversible maturational delay and permanent developmental effects, postnatal studies were initiated. Cystic kidneys developed postnatally in mice whose mother was exposed to TCDD during pregnancy or at the time of birth. The highest incidence occurred in those mouse pups who nursed a mother treated at birth or whose mother was treated during pregnancy and subsequently nursed her young. Mean body weight of litters nursing mothers treated with 10 µg/Kg TCDD at birth was less than that of controls. Absolute and relative thymus and spleen weights were also reduced. No effect on body weights or organ weights were seen in pups whose mother received 3 µg/Kg TCDD at birth. Kidney effects were seen at both dose levels.

MONS 206992

Chlorodibenzo-p-dioxin Toxicology

by: B. A. Schwetz, J. M. Norris, G. L. Sparschu, V. K. Rowe, P. J. Gehring, J. L. Emerson and C. G. Gerbig

Severe toxicological responses have been attributed to certain chlorodibenzo-p-dioxins. Therefore, the acute and subacute toxicities of several of these compounds have been determined in order to provide information useful to the evaluation of potential health hazards.

2,7-Dichlorodibenzo-p-dioxin (2,7-DCDD) was not acnegenic, embryotoxic or teratogenic and was lethal in the g/kg dose range in rats and mice when given as a single oral dose.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) was the most toxic chlorodibenzo-p-dioxin studied, having an LD50 in the µg/kg range in several species of laboratory animals. This chlorodibenzo-p-dioxin was acnegenic, highly embryotoxic and positive for the chick edema factor. The no-effect dose levels for embryotoxicity and chick edema were 0.03 and 0.1 µg/kg/day respectively.

Hexachlorodibenzo-p-dioxin (HCDD) was less toxic than 2,3,7,8-TCDD but was definitely more toxic than either 2,7-DCDD or OCDD (octachlorodibenzo-p-dioxin). HCDD was acnegenic, teratogenic (at 100 µg/kg/day), positive for the chick edema factor and was lethal in the mg/kg dose range in rats given a single dose orally. The no-effect dose levels for embryotoxicity and chick edema were 0.1 and 1.0 µg/kg/day respectively.

OCDD was not acnegenic or teratogenic but was embryotoxic. This chlorodibenzo-p-dioxin was negative for the chick edema factor and was lethal to mice and rats in the g/kg dose range when given as a single oral dose. The no-effect dose level for embryotoxicity was 100 mg/kg/day.

MONS 206993

GENERAL BIOLOGICAL EFFECTS OF TCDD IN LABORATORY ANIMALS

M. Harris, J. Moore and J. Vos

Randombred albino rats (CD stock) weighing 150-200 gm, received 0, 5, 25, 50 or 100 µg/Kg TCDD as a single dose in an acetone/corn oil mixture. At the 100 µg/Kg dose level, death occurred in 6 of 14 rats. The mean survival time was 18.3 days with 5 of 6 death occurring between the 18th and 21st day. One of 14 rats which received 50 µg/Kg died 14 days after treatment. Typically, there is an immediate depression of mean weight gain or a mean weight loss observed at these two dose levels. Animals which die continue to lose weight until death while rats which survive these doses continue to have depressed weight gain. Mean food consumption is less that of controls but never was below 66% of control dietary intake. Toxic clinical signs which were seen at the high dose were ruffled hair coat, hunched posture, inactivity (depression) and jaundice which appeared one to two days preceding death. At the 25 µg/Kg dose mean weight gain is depressed for 14 days after which it becomes equivalent to control values. Weight gain, appearance, and food consumption of rats at the 5 µg/Kg dose were identical to controls.

Daily oral administration of 10 µg/Kg TCDD caused death in 15/16 rats at a mean of 21.8 days after treatment commenced. The pattern of response observed was similar to that seen at the single oral 100 µg/Kg dose. Rats which received 31 daily oral doses at the 1 µg/Kg dose level had depressed weight gains, with the greatest depression seen during the third and fourth week of treatment. Weight gain returned to control levels 7-14 days after TCDD administration ceased.

Death occurred in 9 of 10 female Hartley strain guinea pigs, an average of 18.1 days after receiving 3 µg/Kg TCDD per os. Weekly oral doses at the 1 µg/Kg caused death in 19 out of 19 guinea pigs at a mean of 28 days after treatment commenced. In all cases, a period of marked weight loss preceded death. Animals which received weekly oral doses of 0.2 µg/Kg TCDD began to weigh less than controls during the 4th week and weight gain depression continued through termination of the study at 8 weeks. Similar effects were not observed at the 0.04 or .008 µg/Kg dose.

A single oral dose at 1, 10 or 50 µg/Kg in adult randombred mice (CD-1 stock) had no effect of appearance or body weight. Adult C57Bl/6 mice received weekly oral doses of 25, 5, 1 or 0.2 µg/Kg for four weeks. The 25 µg/Kg dose produced significant weight loss with one mouse death in the 25th day of the study.

MONS 206994

HEMATOLOGIC AND CLINICAL CHEMICAL EFFECTS OF 2,3,7,8-TETRACHLORODIBENZODIOXIN IN LABORATORY ANIMALS

ABSTRACT

J. Zinkl, J. A. Moore, J. G. Vos and B. N. Gupta

Clinical chemical and hematologic parameters were determined in female rats treated daily with 0.1, 1.0 and 10.0 µg/Kg body weight 2,3,7,8-tetrachlorodibenzodioxin (TCDD) for up to 31 days. The concentrations of urea nitrogen, creatinine, glucose, protein, bilirubin, cholesterol, sodium and chloride, and the activities of the enzymes alkaline phosphotase, lactic dehydrogenase, glutamic-oxaloacetic transaminase (S-GOT) and glutamic-pyruvate transaminase (S-GPT) in serum were determined. The most striking changes were increased S-GOT and S-GPT activities. S-GOT activity was elevated after 10 days treatment at 10.0 µg/Kg and after 17 days at 1.0 µg/Kg. S-GPT activity was increased after 13 days in the 10 µg/Kg rats and at 31 days in the 1.0 µg/Kg group. Bilirubin concentration was elevated in the rats treated with 10 µg/Kg after 13 days. Thereafter, it continued to rise, reaching values approximately 10 times the control rats' concentrations at 24 days. Glucose concentration was decreased in all the TCDD treated rats after 10 days treatment, and remained lower than controls throughout the rest of the experimental period.

Platelet depression was found in all TCDD treated rats after 10 days treatment; the degree of depression was dose related. An apparent leukopenia occurred in the 1.0 µg/Kg group after 31 days treatment. Hemoconcentration was noted in moribund animals.

Hematologic parameters were also determined in guinea pigs treated weekly with 1.0, 0.2, 0.04 and 0.008 µg/Kg B.W. The 1.0 µg/Kg dose caused death after 3 to 4 treatments and hematologic parameters were determined on some of these animals at this time. Decreased platelet and leukocyte counts as well as hemoconcentration was observed. A hematogram was assembled on the guinea pigs in the remaining groups after six weeks of treatment. The 0.2 µg/Kg dose groups had decreased platelet and leukocyte counts. No hematologic changes were found in the animals given 0.04 and 0.008 µg/Kg.

Eight week old female CD-1 mice given a single dose of 1.0, 10.0 and 50.0 µg/Kg B.W. TCDD were bled from the retina orbital sinus after 1, 3 and 5 weeks for hematology. Leukopenia was present after 1 week, primarily due to lymphopenia. During the next four weeks, the leukocyte counts increased but were still less than controls after 5 weeks.

MONS 206995

EFFECTS OF TCDD UPON HEMOSTASIS AND HEMATOLOGIC FUNCTION IN THE RAT

Joseph Weisberg and Joseph Zinkl

2,3,7,8-Tetrachlorodibenso-p-dioxin (TCDD) was shown to depress the platelet count and to alter platelet function and coagulant activity in the rat. Daily oral doses of TCDD at a level of 10 ug/kg/day were administered to female rats, and the animals were sacrificed at days 10 and 14 of treatment. Elevations in red cell count, hemoglobin and hematocrit, consistent with hemoconcentration, were observed in the TCDD-treated animals. Moderate leukocytosis, reticulocytosis, neutrophilia and eosinopenia were also observed in the treatment groups. Of interest was a selective depression of the platelet count in treated rats. Platelet function as assessed by platelet aggregation, bleeding time and platelet factor III activity was normal, while clot retraction was abnormal. Prolonged prothrombin and prothrombin consumption times were observed. Serum assays for fibrin degradation products were negative in treated and control groups. The data suggest that under the conditions of this study TCDD results in nonspecific alterations of hematopoietic function, as well as thrombocytopenia, thrombocytoasthenia and derangements in blood coagulation.

MONS 206996

PATHOLOGIC EFFECTS OF TCDD IN LABORATORY ANIMALS

B. Gupta, J. Vos, J. Moore, J. Zinkl and B. C. Bullock

Gross and microscopic examinations were performed on rats, guinea pigs and mice treated with TCDD. Dose ranged from "no effect" to one which produced death; frequency of dose ranged from a single intubation to daily or weekly administration for several weeks. Lymphoid organs (thymus, spleen and lymph nodes) were consistently effected over a wide spectrum of dose ranges in all species examined. Atrophy of the thymus, as denoted by a dose related decrease in weight, was a very sensitive index of TCDD exposure. Histological examination revealed a decrease in cortical thymocytes. Relative lymphoid depletion in spleen and lymph node was also observed.

Liver was the only other organ in which changes consistently occurred. The degree of hepatic involvement seemed dose dependant but severity of change produced at a given dose level was quite variable between species. The severest hepatic effects were seen in rats which received a lethal dose of TCDD. These rats, which were jaundiced, had diffuse degenerative and necrotic hepatic changes. Other changes found inconsistently were hemorrhage in the gastrointestinal tract, heart and brain as well as organized thrombi in various organs. At sublethal dose levels transient degenerative and necrotic hepatic changes were followed by megalocytosis and regeneration.

Although degenerative and necrotic liver changes were also seen in guinea pigs and mice, the magnitude of these effects were markedly diminished. To illustrate this point, hepatic changes produced at lethal TCDD levels in the rats are severe enough to be a contributing cause of death while hepatic changes in the guinea pig receiving a lethal dose are quite mild.

MONS 206997

Ultrastructural Changes in Rat Liver Cells Following a Single Injection of TCDD.

B. Fowler, G. Lucier, H. Brown and O. McDaniel, NIEHS, Research Triangle Park, North Carolina 27709.

Ultrastructural alterations of liver parenchymal cells involving the endoplasmic reticulum and mitochondria are known to occur in animals given TCDD, but the sequence of these changes following administration of TCDD has not been reported. The present study was undertaken to correlate ultrastructural changes in rat liver cells with biochemical studies on liver microsomes and mitochondria. Animals given a single injection of TCDD at doese levels of 5 µg/kg or 25 µg/kg and controls were killed at various times from one to thirty days following the day of administration. Blocks of liver tissue from all animals were processed for both light and electron microscopy. Liver sections of rats given TCDD were histologically indistinguishable from controls. No significant ultrastructural changes could be discerned in liver parenchymal cells until the third day following injection of TCDD. At this time a proliferation of smooth endoplasmic reticulum (SER) was observed around the periphery of parenchymal cells, particularly in areas adjacent to bile canaliculi. This effect was more prominent in animals given the higher dose of TCDD. A mild increase in rough endoplasmic reticulum (RER) was also noted. Moderately swollen mitochondria were occasionally observed in liver cells of animals given 25 µg/kg of TCDD. By seven days, large aggregates of SER were noted in other areas of cells from treated animals. Massive amounts of RER and small numbers of moderately swollen mitochondria were also seen. A gradual decrease in the amounts of SER and RER was observed at subsequent time points.

MONS 206998

EFFECT OF TCDD ON THE IMMUNE SYSTEM OF LABORATORY ANIMALS

J. G. Voa, J. A. Moore, and J. Zinkl

One of the most striking effects of TCDD at sublethal dose levels is atrophy of the thymus. Because of the thymus being the central lymphoid organ for the cell-mediated immunity, function studies of the immune system were carried out in guinea pigs, ratsand mice.

In two experiments, groups of 10 female guinea pigs were doaed weekly for 8 weeks with 0, 0.008, 0.04, 0.2 and 1.0 µg TCDD/Kg body weight to test the cell-mediated and humoral immunity. All animals at the 1 µg/Kg level died or were killed when moribund between daye 24 and 32, showing sevére weight loss, lymphopenia, and atrophy of the lymphoid organs. Cell-mediated immunity was asseaaed by vaccination of the remaining animals with an oil suspension of killed Mycobacterium tuberculosis at day 35, followed by intracutaneous tuberculinstions of the animals at day 47 and 54 with PPD. The diameters of the skin reactions, as a parameter for the cell-mediated (delayed type) hypersensitivity, measured after 24 and 48 hours, were significantly reduced at the 0.2 and 0.04 µg/Kg levels. No significant effect was found on body and adrenal weights at the latter level. Thus, indirect immunosuppression by stimulation of adrenocortical activity was excluded. The humoral immune system was stimulated in a aecond experiment by tetanus toxoid injections at day 28 and 42. Serum tetanus-antitoxin titers of the treated animals, determined with a single radial immunodiffusion technique, were not reduced at day 42, 49 and 56 when compared with the control titers.

A akin test, similar to that in guinea pigs, was done in rats treated weekly for 6 weeks with 0, 0.2, 1.0 and 5.0 µg TCDD/Kg body weight. Animals at the 5 µg/Kg level had significant lower body, thymus and adrenal weights. No effect was found on the skin reactions.

Cell-mediated immunity was teated in mice in a graft versus host assay. Groups of donor mice (C57Bl/6) were dosed weekly for 4 weeks with 0, 0.2, 1.0 and 5.0 µg TCDD/Kg body weight. There was no effect on the body weight. Thymus weights were decreased at the 1.0 and 5.0 µg dose levels. Parental strain (C57Bl/6) apleen cells were injected into the feet of hybrid recipients (C57Bl/6 x DBA-2 F-1). The weights of the draining popliteal lymph node, as a parameter for the graft versus host activity, were significantly lower in the animals injected with spleen cells from the 5 µg/Kg donor group.

From the results, it is concluded that TCDD at sublethal dose levels suppresses the cell-mediated immunity in both guinea pigs and mice. Humoral immunity was not affected in the guinea pigs.

MONS 206999

CARCINOGENESIS BIOASSAY OF CHLORINATED DIBENZODIOXINS AND RELATED CHEMICALS

Maurice E. King and A. M. Shefner
of IIT Research Institute

Richard R. Bates
of National Cancer Institute

The purpose of this study is to determine the chronic toxicity and carcinogenicity of a series of chlorinated dibenzodioxins which can occur as trace contaminants in the manufacture of pesticides and herbicides. Three of the dioxins, 2,3,7, trichloro-, 2,3,7,8, tetrachloro-, and the hexachloro derivatives are extremely toxic and require the use of special containment and animal holding facilities.

Groups of B6C3F1 mice and Osborne-Mendel rats are receiving the unsubstituted, 2,7-dichloro-, and the octachloro dioxins at levels of 1% and 0.5% of their diet. These feeding studies will continue for a total of 90 weeks with mice and 110 weeks with rats and will be initiated with the toxic derivatives when they are available.

Chronic skin carcinogenesis studies to assess the activity of dioxins as complete carcinogens and as promoting agents have been in progress for over a year. In the complete studies the compounds are dissolved in acetone and applied to the backs of Swiss/Webster mice three times a week. For the promotion experiments, each mouse was initially treated with 50 μg DMBA and one week later application of the test compounds was begun.

Skin tumor formation has been observed in only one female mouse treated with octachlorodioxin in the complete study. None of the other dioxins being assayed in this manner have produced papillomas on any of the mice. The promotion studies however have yielded papillomas on 15 males treated with unsubstituted dibenzo-dioxin and 23 males treated with acetone. Surprisingly dioxane is as effective in promotion in producing papillomas and carcinomas, (on males and females) as croton oil which is used as a positive control. In view of the wide-spread use of dioxane this result may be of significance.

CHLORINATED DIBENZODIOXINS AND PENTACHLOROPHENOL

ABSTRACT

R. L. Johnson, P. J. Gehring, R. J. Kociba

Dow Chemical U.S.A.

Commercial grades of pentachlorophenol have been found to contain as high as 2500 ppm chlorinated dibenzo-p-dioxins. Hence, there has been concern about the potential hazard of this chlorinated phenol in view of its widespread use as a wood preservative.

The predominant dioxin in commercial pentachlorophenol is octachlorodibenzo-p-dioxin which appears to be the least toxic member of this class of compounds. Rats survive a single dose of 1 g/kg of this compound while doses of less than 100 µg/g of the most toxic member, 2,3,7,8-tetrachloro-dibenzo-p-dioxin, are lethal. The latter compound has not been found in commercial pentachlorophenol at levels greater than 0.05 ppm which is the lowest detectable level using current analytical procedures.

In a series of toxicological evaluations of commercial grades of pentachlorophenol in animals it was found that some untoward effects were associated with the chlorinated dibenzo-p-dioxin content. These included chloracne activity

and production of chick edema as well as histopathological alterations in rats fed diets containing the material for 90 days. Purified pentachlorophenol, 99.9% pure, did not produce these effects. Neither purified nor commercial grades of pentachlorophenol had a teratogenic effect when administered to pregnant rats.

In order to minimize the hazard associated with chlorinated dibenzo-p-dioxins in commercial pentachlorophenol, a new production process was developed capable of producing a new pentachlorophenol with significantly lower chlorinated dibenzo-p-dioxin content. The new pentachlorophenol mimics pure pentachlorophenol in its toxic properties.

This new product, which is now registered under the Federal Insecticide, Fungicide and Rodenticide Act, has been extensively evaluated--and is continuing to be extensively evaluated--to assure that the low levels of chlorinated dibenzo-p-dioxins are toxicologically insignificant. Thus far, none of the previously referred to untoward effects of chlorinated dibenzo-p-dioxins have been produced with this product. Indeed, a sample of pentachlorophenol containing two-fold greater amounts of chlorinated dibenzo-p-dioxins has failed to produce toxic manifestations characteristic of these materials.

bak
1/16/72

ACUTE AND CHRONIC TOXICITY OF 2,3,7,8-TETRACHLORDDIBENZO-p-DIOXIN (DIOXIN) IN AQUATIC ORGANISMS

by

R. A. Miller, L. A. Norris, and C. L. Hawkes

ABSTRACT

The herbicide 2,4,5-T is an important tool for the control of undesirable hardwoods which prevent rapid reforestation of cutover forest lands. Residues of 2,4,5-T ranging from less than 0.5 to 20 ppb are found in forest streams which flow through or nearby areas receiving aerial applications of the herbicide. Herbicide residues approaching 1 ppm may result when direct application to stream surfaces or marshy areas occur. We assume direct application of 2,4,5-T to stream surfaces will also introduce dioxin. If the herbicide contains 0.5 ppm dioxin, we expect dioxin residues in streams ranging from less than 0.00025 ppt to about 0.01 ppt after carefully controlled aerial applications of 2,4,5-T. Dioxin concentrations near 0.5 ppt may occur when marshes or streams receive direct applications of 2,4,5-T. The toxicity of 2,4,5-T in several aquatic organisms is known, but information on the toxicity of dioxin to aquatics is lacking.

We exposed guppies and coho salmon fingerlings to various concentrations of dioxin ranging from 0.056 ppt to more than 0.2 ppb for 24, 48, and 96 hours. Fish were then transferred to fresh water and weight and mortality tallied. Characteristically there was little

MONS 207004

or no mortality during the exposure period and for several days after removal to fresh water. At concentrations greater than 5 ppt, however, complete mortality occurred during the succeeding 60 days. The duration of the exposure period was not nearly as important as initial concentration in determining mean survival time. Dioxin was more toxic to small fish than to large fish of the same species. The growth of salmon fingerlings was markedly reduced after exposure to dioxin. Observations of treated fish revealed a concomitant drop in food consumption. The threshold response level for 24-, 48-, or 96-hour exposure to dioxin appears to be between 0.056 and 0.56 ppt for coho salmon fingerlings.

We are examining now the toxicity of dioxin in rainbow trout exposed through their food. The animals are receiving 1,000, 1, or 0.001 nanograms dioxin per gram of fish (fresh weight) per week. The dioxin is contained in a prepared fish food at concentrations up to 2 ppm. Fish take dioxin containing food until the desired daily dose has been consumed and then additional dioxin-free food is offered. Survival and growth of fish are tallied at regular intervals through the test period.

In other tests, mosquito larvae, oligochaete worms, and pulminator snails were maintained in water which originally contained 0.2 ppb dioxin for 5, 6, or 6 weeks, respectively. Water was not changed

and fresh dioxin was not added during this period. These aquatic organisms were less sensitive than fish to dioxin. The pupation of mosquito larvae and adult emergence were not affected. There were little or no effects on the numbers, total biomass, or mean weight of oligochaete worms. There was no toxicity among adult pulminator snails. Snail reproductive success is still under study.

Acute and chronic oral toxicity of chlorinated dibenzofurans to salmonid fishes

V. Zitko and D.J. Wildish
Environment Canada Fisheries Reaearch Board
Biological Station, St. Andrews, New Brunswick

O. Hutzinger
Atlantic Regional Laboratory, National
Research Council of Canada, Halifax, Nova Scotia

and

P.M.K. Choi
McMaster University, Hamilton, Ontario

A median mortality of 120±30 days occurred among juvenile Atlantic salmon (*Salmo salar*) fed dry fish food contaminated with a mixture of chlorinated dibenzofurans to contain 2.7, 5.7, 2.8, and 9.1 µg/g wet weight of di-, tri-, tetra-, and octachlorodibenzofuran, respectively. Only octachlorodibenzofuran at levels of 0.03 and 0.2 µg/g wet weight in muscle and gut, respectively, was detected in dead fish. The corresponding levels of this compound in fish which survived 140 days of feeding were 0.01 and 0.02 µg/g wet weight, respectively. Experiments to determine acute oral toxicity of dichlorodibenzofuran to brook trout (*Salvelinus fontinalis*) are under way.

MONS 207007

The Search for Chlorinated Dibenzofurans and Chlorinated Dibenzodioxins in Wildlife Populations Showing Elevated Levels of Embryonic Death

Gerald W. Bowes[1], Bernd R. Simoneit[2], A.L. Burlingame[2], Brock W. de Lappe[3], David B. Peakall[4], and Robert W. Risebrough[1]

[1]Toxic Chemicals Section,
Canadian Wildlife Service,
Ottawa, Ontario K1A 0H3

[2]Space Sciences Laboratory,
University of California at Berkeley,
Berkeley, California 94720

[3]Bodega Marine Laboratory,
University of California,
Bodega Bay, California 94923

[4]Section of Ecology and Systematics,
Cornell University,
Ithica, New York 14850

Local populations of several species of fish-eating birds in North America have shown a decrease in reproductive success resulting from the death of unhatched embryos. In the laboratory, embryonic deaths have also been recorded among birds treated with PCB and have been attributed to chlorinated dibenzofuran contaminants in the commercial preparation. The wild populations also occasionally show birth defects comparable to those produced in the laboratory by the chlorinated dibenzodioxins. In southern California, the sea lions (Zalophus californianus)

are currently showing an elevated incidence of premature birth and death of the pups. The PCB concentrations in the wild populations of both birds and sea lions are among the highest recorded in the environment. The hypothesis that the abnormalities are related to the chlorinated dibenzofurans or chlorinated diobenzodioxins is explored with high resolution mass spectrometry.

The halogenated organic matter was prepared for mass spectrometeric analyses by extraction and partial clean-up in carbon tetrachloride and sulfuric acid, clean-up on Florisil, and fractionation on Florisil and alumina columns. With the use of electron-capture gas chromatography, a further fractionation was made, and components were collected in the retention time range of the chlorinated dibenzofurans and chlorinated dibenzodioxins. The fractions were re-chromatographed on electron-capture g.c. and collected again using a longer preparatory column. The concentrated fractions were subjected to online mass spectrometry using direct introduction into the ion source.

Samples examined included tissues of aborting sea lions from southern California and dead embryos of the herring gull (Larus argentatus) from the Great Lakes and of the osprey (Pandion haliaetus) from Long Island Sound.

Studies on TCDD-Induced Changes in Rat Liver Microsomal and Mitochondrial Enzymes

G.W. Lucier, O.S. McDaniel, B.W. Fowler, E. Faeder, G. Hook, and B.R. Sonawane

Male rats were administered a single oral dose of TCDD (5 or 25 µg/kg) and time-course measurements made on some hepatic microsomal and mitochondrial enzymes. Cytochrome P-450 contents were significantly induced at all sampling periods (1-28 days) with either dose. Maximum induction (2-fold) occurred between 3 and 10 days after treatment. Cytochrome b_5 contents were increased by the same magnitude and followed the same time-course as P-450. Hydroxylation of aniline was induced by TCDD treatment and aminopyrine demethylation rates were decreased. Enzyme activities were calculated per mg microsomal protein and in general microsomal protein contents were increased by approximately 15%. Studies were also performed on benzphetamine demethylation, benzpyrene hydroxylation, hydroxylations of biphenyl at the 2- and 4- positions, TPNH cytochrome C reductase, β-glucuronidase, and acid phosphatase.

The most striking induction occurred with UDP glucuronyltransferase, which was induced approximately 8-fold on days 6, 9, and 16. Enzyme activity was still elevated 3-fold twenty eight days after TCDD-treatemnt. Induction of glucuronyltransferase was studied in reference to enzyme kinetics, microsomal activities in smooth- and rough- surfaced endoplasmic reticulum, microsomal phospholipid content, activation phenomenon, and induction in extraheptic tissues. Possible hepatic necrosis, during the time-course experiment, was monitored by standard serum tests.

Oxidative phosphorylation rates in rat liver mitochondria, using succinate as the substrate, were unchanged at both dosages and all sampling periods although prepared mitochondria from TCDD-treated rats appeared to be more unstable at 4° C than mitochondria from control rats.

NIEHS CONFERENCE ON

CHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

PROPOSED PRESENTATION

ABSTRACT

The toxic action of 2,3,7,8-tetrachlorodibenzodioxin in rats is very prolonged, with deaths occurring as long as 15 weeks after a single oral dose. However changes in liver composition and metabolism of drugs take place within 24 hr of dosing. A divergent effect on the metabolism of zoxazolamine and hexobarbitone has been shown in vivo. The degree of this effect appears to follow the morbidity of the animals. The corresponding in vitro changes are described and it is shown that there are drastic alterations in the components of the drug-metabolising system of liver microsomes. Alterations in the spectral properties of cytochrome P_{450} and its interaction with endogenous and exogenous substrates will be discussed.

J.B.GREIG

MONS 207011

STUDIES OF THE EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN ON MAMMALIAN HEPATIC δ-AMINOLEVULINIC ACID SYNTHETASE

James S. Woods

National Institute of Environmental Health Sciences
Research Triangle Park, N. C. 27709

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a toxic contaminant associated with the synthesis of the herbicide 2,4,5-trichlorophenoxyacetic acid, polychlorinated biphenyls and other chlorinated hydrocarbons. Recent studies have indicated that TCDD is a potent inducer of hepatic δ-aminolevulinic acid (ALA) synthetase in chick embryos, and it is suggested that prior exposure of humans to TCDD may promote the incidence of porphyria cutanea tarda resulting from induction of hepatic ALA synthetase. In order to evaluate the potential prophyrogenic effects of TCDD in mammals, hepatic ALA synthetase activity was measured in male rats at periods up to 30 days after the oral administration of 5, 25 or 100 μg/kg doses of TCDD. At no dose level tested did the hepatic ALA synthetase activity differ from control levels, as measured in both liver homogenates and subcellular fractions. Furthermore, there was no porphyrin accumulation in livers of TCDD treated rats as indicated by the absence of fluorescence. TCDD treatment did not alter the subcellular distribution of the enzyme activity seen in control animals and had no direct effect on the activity of ALA synthetase purified from rat liver when incubated with the enzyme at concentrations up to 10^{-6}M. Similar observations were made after treatment of mice and guinea pigs with TCDD. These studies indicate that TCDD is not porphyrogenic in mammals even when administered at several times the reported LD_{50} dose level. These studies further document the marked differences observed in the response of different species to various drugs and chemicals.

MONS 207012

THE EFFECT OF TCDD ON BILIARY EXCRETION OF INDOCYANINE GREEN

Shang W. Hwang

Male randombred CD rats, weighing 350-400 gm, received a single oral dose of 25 µg/kg or 5 µg/kg TCDD in an acetone corn oil mixture. Control rats received an equivalent dose of acetone and corn oil. At day 1, 7 and 16 after treatment, the effect of TCDD on bile flow and biliary excretion of indocyanine green (ICG) was examined. Animals were anesthetized with pentobarbital Na (50 mg/kg); the renal pedicles were ligated and the common bile duct cannulated; body temperature was maintained at 37°C. Bile was collected for a 20 min period and the volume measured by weighing. The bile flow rate increased the first day after TCDD treatment and continued to increase through the 16th day. The rats receiving 25 µg/kg had the highest flow rate. After measuring flow rate, 6.25 mg/kg ICG was injected i.v. to each rat and bile collected for another 20 min. A blood sample was withdrawn and liver removed at the end of bile collection. Both the concentration of ICG in bile and the total ICG excreted in bile during the 20 min period significantly decreased after TCDD treatment. The 25 µg/kg dose caused more inhibition of ICG excretion in bile and more retention of ICG in blood. The rate of ICG disappearance from plasma was also determined by measuring the dye concentration in series of plasma samples which were withdrawn at 1, 2, 5, 8, 12 and 20 min after ICG injection. The disappearance rate decreased with increasing TCDD dose with the greatest decrease in disappearance rate seen at the 16th day at the 25 µg/kg dose. In contrast to the plasma level, the 5 µg/kg rats accumulated more ICG in liver than 25 µg/kg rats. Control rats showed the lowest ICG liver level. TCDD also caused an increase in liver weight which was dose related.

Chlorinated Dibenzo-p-dioxin Distribution Within Rat Tissues And Subfractions of the Liver*

D. H. Norback, J. F. Engblom and J. R. Allen†

One hundred µg of Cl^{36}-octachlorodibenzo-p-dioxin dispersed in corn oil was administered to male rats by gastric intubation daily for 21 days. Digested samples of urine, feces, serum, selected organs and subcellular hepatic fractions were analyzed for radioactivity with a liquid scintillation counter. Ninety-five percent of the total dose was recovered in the feces and over 4% was present in the urine. After 21 days, less than 0.3% of the total administered dose was detected in the total rat tissues. The liver contained the highest concentration per unit weight with adipose tissue containing concentrations 1/3 as great as that present in the hepatic tissue. Significant levels of radioactivity were found in the kidneys, heart, reproductive organs, skeletal muscle, skin and serum. The greatest reservoirs of the compounds per organ were liver and skin. Over 95% of the radioactivity within the liver was associated with the microsomes with equal distribution within the rough and smooth fractions. Radioactivity of the urine was confined to the lipid soluble portion. Distribution of the radioactivity within rats that subsequently received a control diet for 7 weeks was confined to the liver, adipose tissue and skin at levels of approximately 20% that present in the tissues of rats following 21 days of uninterrupted ingestion.

* Supported in part by U.S. Public Health Service grants ES-00472 and RR-00167 from the National Institutes of Health.

† Department of Pathology, University of Wisconsin Medical School, and Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin 53706.

MONS 207014

Abstract: Studies on the Mechanism of Action of the Halogenated Dibenzo-p-dioxins

Alan Poland, Ed Glover - University of Rochester, School of Medicine and Dentistry, Department of Pharmacology, Rochester, New York 14642

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is perhaps the most potent small-molecule toxin and teratogen known. This starting point of our work was the observation that workers in a factory producing 2,4,5-T daveloped chloracne and porphyria cutanea tarda (Bleiberg et al., Arch Derm 89, 793, 1964) and both conditions remittad following the institution of methods to reduce TCDD contamination. Hepatic porphyria is produced by many xenobiotics, all of which have in common the ability to stimulate the initial anzyme in hepatic heme formation, δ-aminolevulinic acid synthetase (ALAS).TCDD produced a dose-related stimulation of ALAS, in the chick embryo liver; at 4.66×10^{-12} moles/egg (1.5 ng) thare was a doubling of enzyme activity and at 1.55×10^{-9} moles/egg (0.5 µg) there was over a 35-fold stimulation of ALAS activity. Unlike other drugs which stimulate ALAS, induction with TCDD is prolonged in time. Testing a series of 15 dioxins for their ability to induce ALAS gave a sharp structure-activity relationship (SAR); and only those congeners possessing at least 3 halogen atoms in the 2,3,7 and 8 positions, and at least one non-halogenated carbon atom were inducers. The ability of the isomeric dioxins to stimulate ALAS correlates very well with their toxicity or ability to produce acne.

We next studied the effect of TCDD on aryl hydrocarbon hydroxylase (AHH) activity, because of the close relationship between the induction of ALAS and microsomal oxygenase AHH was induced in chick embryo liver by as little as 1.55×10^{-12} moles/egg (0.5 ng), and induction by various dioxin congeners gave an identical SAR as with induction of ALAS. In the rat, TCDD was also shown to be a potent inducer of AHH; with half-maximal stimulation at $\sim 8 \times 10^{-10}$ moles/kg (.26 µg/kg, about 1/100 the LD_{50} dose). TCDD is $2.8 \times 10^{4} \pm 1.38$ times as potent as methylcholanthrene at inducing AHH in the rat. A single dose of TCDD stimulates hepatic AHH and cytochrome P-450 for more than 35 days in the rat.

A mechanism of toxicity of TCDD is proposed and its implications discussed. We suggest the chemically inert parent compound is not the toxic moiety, but rather cell damage is dependent on metabolism of TCDD to a high reactive intermediate.

MONS 207015

UNIVERSITY OF WISCONSIN—MADISON

DEPARTMENT OF ENTOMOLOGY

237 Russell Laboratories
1630 Linden Drive
Madison, Wisconsin 53706

Abstract

Two approaches were made to the problem of determining the environmental importance of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The first was to determine the ability and level of microbial degradation, and the second approach to examine the biological affinity or bioaccumulation of TCDD in a number of artificial ecosystems. A series of microorganisms which have shown an ability to degrade other chlorinated hydrocarbons were tested for their ability to degrade TCDD. Most of these organisms showed no ability to metabolize TCDD, though a few exhibited a limited degree of degradation. These organisms are being more intensively studied. In the second approach we have studied the rate of bioaccumulation in several model ecosystems. Attempts were made to correlate the solubility, migration, biological affinity etc of TCDD with a number of reference pesticides (DDT, zectran, γ-BHC). In the first model system we studied the ability of TCDD to move from sand and into organic soil. DDT showed a much greater ability to leach into soil than did TCDD. The second model ecosystem involved biological partitioning, where TCDD and other chemicals were first impregnated into yeast or algae, and the rate of absorption of TCDD by aquatic invertebrates were examined. In the third model ecosystem TCDD and other chemicals were first applied to dry sand particles. The coated sand was introduced into aquaria where various organisms were reared. The extent of biological accumulation in these organisms were studied. In all cases TCDD had the lowest biological affinity, whereas DDT was quickly picked up by biological systems. The pesticides with high water solubility (γ-BHC, zectran), on the other hand, showed lower degrees of bioaccumulation than DDT.

Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachlorobenzo-p-dioxin

by
Fumio Matsumura and H. J. Benezet
Department of Entomology, University of Wisconsin, Madison

COLLEGE OF AGRICULTURAL AND LIFE SCIENCES

MONS 207016

ENVIRONMENTAL GENERATION AND DEGRADATION OF DIBENZODIOXINS AND DIBENZOFURANS

D. G. Crosby, K. W. Moilanen, and A. S. Wong
Department of Environmental Toxicology
University of California, Davis, California 95616

Toxic dibenzodioxins and dibenzofurans occur as trace impurities in widely-used chlorophenols and their derivatives. They arise under the drastic conditions employed in manufacture; both free radical and nucleophilic displacement reactions probably are involved. There is evidence that they also may be formed by pyrolysis.

Even under mild conditions, light can promote reactions in otherwise unreactive aryl halides; for example, aryl halides form phenols by photonucleophilic displacement. Irradiation of ortho-chlorophenols could be expected to produce dibenzodioxins but, while pentachlorophenol indeed provides octachlorodibenzodioxin (OCDD), the 2,3,7,8-tetrachloro analog (TCDD) is not detected in the photolysis of 2,4,5-trichlorophenol. Chlorinated dibenzofurans similarly may result from polychlorinated biphenyls.

However, both the chlorinated dibenzodioxins and dibenzofurans are very unstable to light in the presence of organic substrates. While they could be generated under environmental conditions, light provides a mechanism for their rapid destruction. That physical forces unexpectedly may generate or degrade toxic substances in the environment demonstrates the extreme importance and close relation of xenobiotic chemistry to environmental health.

MONS 207017

PHOTOCHEMICAL DEGRADATION OF DI- AND OCTACHLORODIBENZOFURAN

O. Hutzinger, S. Safe — Atlantic Regional Laboratories, NRCC, Halifax, Nova Scotia, Canada

B.R. Wentzell — Chemistry Dept. Acadia University Wolfville, Nova Scotia, Canada

V. Zitko — Environment Canada, Fisheries Service St. Andrews, New Brunswick, Canada

The photochemical behaviour of 2,8-dichlorodibenzofuran and octachlorodibenzofuran was investigated. These compounds were chosen as representatives (one of low and one of high chlorine content) of a class of compounds which has been considered as potential environmental hazard. Irradiations have been carried out in hexane and methanol solutions in a photochemical reactor at 310 nm. Thin films of the compounds, in quartz tubes, have also been exposed to summer sunlight for several months.

The results indicate that chlorodibenzofurans decompose rather readily in solution. Octachlorodibenzofuran in methanol solution, for example, disappears completely after 30 minutes exposure to light of 310 nm. Chlorobiphenyls decompose much slower under similar conditions. Judging from these preliminary experiments, it would appear that accumulation of chlorodibenzofurans, which may be formed from chlorobiphenyls by photochemical reaction in the environment, is not a likely process.

The products obtained on irradiating di- and octachlorodibenzofuran include compounds which are formed by reductive dechlorination as well as polar materials. The structure of these compounds will be discussed.

MONS 207018

Tetrachlorodibenzodioxin in the Environment: Sources, Fate and Decontamination

P. C. Kearney, E. A. Woolson, A. R. Isensee and C. S. Helling

Pesticide Degradation Laboratory

Agricultural Research Service

United States Department of Agriculture

Beltsville, Maryland 20705

Abstract

A major research effort was initiated in the Agricultural Research Service, U.S. Department of Agriculture to investigate the inputs of and major biological processes affecting 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). A chemical survey of the major phenolic pesticides indicated the herbicide 2,4,5-T[(2,4,5-trichlorophenoxy)acetic acid] contained TCDD. Between 1960 and 1969, the total U.S. production of 2,4,5-T was 106.3 million pounds. The level of input of TCDD on a yearly basis differed with the manufacturer, but ranged from nondetectable (<0.1 ppm) to 40 ppm. Environmental studies indicated that TCDD does not leach in soils, is not taken up into the economic portion of plants growing on contaminated soils, and is degraded to about 50% of the initial concentration after one year in soils and was not detected in soils which received 947 lb/acre of 2,4,5-T 2 to 5 years after application. TCDD is not formed by microbial or chemical condensation of 2,4,5-trichlorophenol in soil.

MONS 207019

Several options are available for the decontamination of surplus stocks of 2,4,5-T containing TCDD. These include incineration, soil biodegradation, chemical conversion to carbon tetrachloride, and purification for use as a herbicide. The feasibility and environmental implications of each are currently under investigation.

For NIEHS Conference Chlorinated Dibenzodioxins and Dibenzofurans April 2-3, 1973.