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Summary of Dioxin Planning Conference
Mayflower Hotel, Washington, D.C.
July 25-26, 1974

INTRODUCTION

A Dioxin Planning Conference was held at the Mayflower Hotel in Washington, D.C. on July 25-26, 1974. Dr. Leonard R. Axelrod (EPA-OPP C&E Div.) at the outset stated the groud rules that would be followed during the course of the meeting. They were as follows:

1. The meeting was held primarily for those parties to the 2,4,5-t hearings.
2. The meeting would be specifically on "dioxins" with emphasis on tetrachlorodioxins (TCDD).
3. The Workshop sessions would be with reference toward data analysis and data retrieval. No discussions were to be made with reference to money and/or to set collaborative agreements.

SESSION I - Analytical Methodology, Thursday, July 25, 1974

Dr. Ralph Ross (EPA-OPP/CED, Washington, D.C.) presented an overall summary of the dioxin project within the C&E Division. Included in his discussion were the underlying reasons for the initiation of the project; the laboratories that have been involved with analyses which included government, industry and academia; the difficulties which have been encountered in the analytical methodology since the project began; the emphasis of the meeting for all parties concerned that some agreement as to the exact methodology to be used for TCDD analysis should be agreed upon.

Dr. Aubry Dupuy (EPA, Mississippi Test Facility, Mississippi) presented the methodology involved in preparing samples prior to mass spectral analysis. Since EPA adopted the modified Baughman-Meselson cleanup procedure for environmental samples, Dr. Dupuy summarized some of the basic difficulties which have been encountered by MTF. He indicated that the procedure is basically suitable for samples from bee, sheep, goat and fish. However, for water ana soil samples the saponification and chromatography step may be altogether eliminated.

Limitations to the method included samples with high carbohydrate content such as rice, hay and grasses. Dr. Dupuy stated that the MTF had been collaborating with EPA-NERC/PTSEL in Research Triangle Park, N.C., and together the two laboratories have developed methodology for extracting TCDD from rice which, at the present time, appears to show no obvious interferences when the samples are analyzed by mass spectrometer for TCDD.

Dr. Taylor and Dr. Hughes (USAF, Aerospace Laboratories, Ohio) presented some GLC-MS work on dioxin content in Herbicide Orange. The data presented was in the ppb range which is out of the sensitivity range as required by EPA for environmental samples.

Dr. David Firestone (FDA, Washington, D.C.) discussed FDA's ESR work on multiple cation radicals of dioxins and stated FDA's interest in pursuing this work further. FDA is currently engaged with control analyses of the Baughman-Meselson cleanup procedure. Some data were given on the efficiency of various alumina types and the different eluting patterns involved for effective cleanup. He also indicated that FDA would be involved in the development of a neutral cleanup technique along with Dr. Meselson's laboratory. Recovery of a number of dioxins will be investigated using this procedure.

Dr. Edward Oswald (EPA-NERC/PTSEL, Research Triangle Park, N.C.) stated PTSEL's involvement in characterization of environmental substrates with no particular emphasis on TCDD. PTSEL is currently looking into other screening procedures for environmental pollutants such as the new Hall detector, a GLC method.

Dr. Oswald pointed out PTSEL's success in developing a procedure for the analysis of TCDD from rice samples. He also implied that the interference problem in analyzing tissue samples for TCDD using high resolution mass spectrometry had not been resolved. PTSEL is currently in the initial stages of developing a GLC-MS method for dioxin analysis.

Dr. Rudy Stehl (Dow Chemical Company, Midland, Michigan) presented some GLC-MS data of some environmental samples which has been analyzed for TCDD. He stated the following.

1. The limits of detection of the technique depended primarily on the type of sample being analyzed.
2. There are no interference problems with samples with dioxin concentrations of 10 ppt and above.
3. GLC-Low resolution MS is adequate for routine analysis (detection limits depend on sample size). Depending on sample type, detection as low as 0.5 ppt have been obtained.
4. GLC-High resolution MS should be available when interference problems are encountered with low resolution (and vice versa). The types of interferences incurred by the two techniques, (high and low resolution MS) are not the same.

Dr. Steve Olin (Tracor-Jitco, Rockville, Md) presented work Tracor-Jitco has contracted for the National Cancer Institute. Dr. Olin gave a brief summary of the various dioxins which were of current interest to NCI-e.g., tetra-, hexa-, and octadioxins. He indicated that Tracor-Jitco was not analyzing these compounds, but that analytical methodology employing a GLC screening method was being conducted by Midwest Research Institute.

Session II - Toxicology, Thursday, July 25, 1974

A 28 week subacute intubation study using 2,3,7,8 TCDD is in progress at IITRI, Chicago, Illinois, according to Mr. William Burnam, Criteria and Evaluation Division, OPP, EPA. Male and female Sprague-Dawley rats were intubated twice weekly with total doses of 1.0 ug/kg/wk, 0.1 ug/kg/wk and 0 ug/kg/wk which approximate 3 ppb, 0.3 pbb and zero TCDD in the rat's daily diet. Rats were killed, histopathologically examined and tissues saved for TCDD analysis at 2,4,8, 16 and 28 weeks. Currently rats are undergoing a withdrawal period and will be killed at 4 and 12 weeks of this period.

It is hoped, that after TCDD residue data become available, the onset or disappearance of pathological changes in various tissue can be related to the TCDD levels in the same tissues.

There is quite a difference between controls and treated rats in liver weight as a percent of body weight at 28 weeks. Values for males at 0.1 ug/kg/week were 35.5% higher than controls and at 1.0 ug/kg/week were 20.4% higher than controls. Values for females at 0.1 ug were 13.7% higher than controls while at 1.0 ug they were 7.4% greater than controls. At each of the previous sacrifice periods, the liver weights for tested rats have been higher than that of the controls but generally the 1.0 ug group liver weight percent of body weight exceeded those of the 0.1 ug group. None of the data have been statistically analyzed.

Preliminary histopathological examination of the 28 week rats shows that the major changes are in the liver of the males. There is centrilobular vacuolization mainly in the males receiving the high dose but some vacuoles were noted in males receiving the low dose. The nature and severity of these lesions has not yet been determined.

In order to make animals receiving subacute dose of 2,3,7,8 TCDD available to other interested researchers, a small number of male and female rats exposed for 28 weeks to TCDD were sent to EPA, NERC, RTP, North Carolina. These were rats receiving either 1.0 ug/kg/wk, 0.1 ug/kg/wk or 0 ug/kg/wk TCDD in twice weekly intubations at IITRI, Chicago, Ill. Dr. Anthony Colucci, N.E.R.C., ORD, EPA, reported that preliminary examination of livers show dark areas on the female liver at both the high and low doses indicative of porphyria. Dr. Joyce Goldstein is currently analyzing these livers for those enzymes which would account for increased porphyria, e.g. ALA synthetase. In both male and females, the liver weights were increased and the thymus weights were decreased at the high dose. Blood will be analysed in a SMA-12 analyzer. No pathological examinations using the light or electron microscope have yet been performed, but these are scheduled for completion in the near future

The National Center for Toxicological Research is currently analyzing data obtained from a large dose-response teratology study with two grades of 2,4,5-T. In addition, Dr. Joseph Holson, Teratology Section, stated that work is planned with the 2,3,7,8 TCDD in teratology and pharmacodynamics areas with mice and rabbits. This will involve a dose-response study which observes cleft plates and kidney development

postnatally as its main area of interest. Research with TCDD will not start until N.C.T.R. can obtain enough TCDD and the analytical capability to detect low levels of TCDD in the tissues.

Dr. Samuel Shibko, FDA, reported that Dr. Tom Collins is involved in teratology studies in hamsters using various pure dioxins. Dr. Green is collecting data on the mutagenic activity of TCDD in an in vivo cytogenic analysis of bone marrow. Even after a 29 day latent period, TCDD has shown no mutagenic effects in rats. Further study is planned in guinea pigs.

Work on the dioxins, mainly 2,3,7,8 TCDD and hexachlorodioxins, is continuing in pharmacology and chemistry at N.I.E.H.S. according to Dr. James McKinney. There is much interest in a radioactive immunoassay method for detecting very low levels of dioxins and this work is ongoing. There is some indication that a metabolite of TCDD is found in the feces. Pharmacodyamic rodent data has indicated little transplacental passage of TCDD. Additional articles should be published soon regarding TCDD's effect on the cellular immune response.

Dow Chemical Company completed a 13 week feeding study including a 7 week pharmacodynamic study in male and female rats intubated with 5 times a week dose of either 1.0, 0.1, 0.01, 0.001 or 0 ug/kg/day of 2,3,7,8 TCDD. The residues levels followed a first order equation. The total body half life using ^{14}C TCDD was approximately 30 days as opposed to a value of 17 days obtained with a single large dose of TCDD. A steady state was approached in the liver and fat. Males and females had similar distribution and excretion patterns. There was good correlation between the ^{14}C TCDD and the unlabled TCDD studies. At the 1.0 ug/kg/day dose there was a possible, as yet unidenified, metabolite.

With regard to overt toxic effects, there were some deaths at 1.0 ug/kg/day. At 1.0 ug/kg/day, there was edema, degeneration of the liver, increased S.E.R., multi-nucleated hepatocytes, increased porphyrin excretion and lymphoid depletion. At 0.1 ug/kg/day, there was increased S.E.R., some vacuolization, increased porphyrin excretion in the females and decreased thymocytes. At 0.01 ug/kg/day there were minor liver changes, but both the two lower doses of 0.01 and 0.001 were essentially comparable to controls. Chief investigators in the Dow study were Drs. Perry Gehring and R.J. Kociba.

Tracor-Jitco is handling various contracts of NCI's bioassay program. At present, intubation or feeding and skin painting studies on rodents are on the following approximate time schedules provided by Dr. Steven Olin.

Unsubstituted dioxin	- Completed
2,7 dichlorodioxin	- Completed skin painting and cancer promotion study; oral to rats and mice almost finished.
trichlorodioxin	- Contains 2,3,7,8 TCDD; currently working on obtaining a pure product
2,3,7,8 TCDD	- Almost finished a 90 day subacute intubation range-finding study for oral studies; have determined a maximum tolerated dose for skin painting in mice.
Hexachlorodioxin	- Work is planned with 1,2,3,6,7,8 and 1,2,3,7,8,9 isomers
Octacholorodioxin	- Increased mortality due to contamination by hexachlorodioxins has put this project off-schedule. Work will begin using octachlorodioxin having no hexachlorodioxin impurities

SESSION III - Monitoring and Residues, Thursday, July 25, 1974

Mr. Allan Crockett discussed 1974 monitoring studies conducted by EPA. These included sample collections in rights of ways, forests and aquatic areas adjacent to rice fields. In all cases, these areas had a history of recent 2,4,5-T usage. Some of the samples from these areas gave tentative but unconfirmed positive analyses for TCDD. This included several species birds and mammals collected from both forests and right-of-way. Out of 13 shrew samples collected from treated right-of-ways, 11 contained detectable levels of TCDD. Analyses for positive samples ranged from 54 to 397 ppt, averaging 202 ppt. For comparative purposes, "control" shrews collected from areas having no pesticide treatment history will also be analyzed. Out of fifteen wild catfish samples collected from different rice growing areas in Arkansas and Mississippi, two gave positive indication of TCDD residues. Further work in these areas of TCDD monitoring will be delayed pending confirmation of these positive TCDD analyses by appropriate confirmatory methodology.

Mr. Collier discussed other EPA studies related to buildup and or decline of TCDD residues in animal tissues. A fish residue study was described wherein bluegill (Lepomis macrochirus) were continually exposed in a flow through system to TCDD at a constant level of 0.1 ng/liter. Samples were collected at 0, 7, 14, 21 and 28 days. Similar samplings were made after transfer into dioxin free water at withdrawal periods of 7 and 14 days.

A Texas rangeland experiment was also conducted wherein cattle, sheep and goats were allowed to graze on range grass freshly treated with the herbicide 2,4,5-T for a period of 39 days. TCDD residues in the fat of these animals ranged from 6 to 31 ppt and in the liver from 1 to 5 ppt.

Treatment level was at 1/2 lb. active ingredient per acre and TCDD content of the formulation was about .04 ppm.

EPA analyses of fat samples from a Dow calf feeding study were also described. Beef calves fed for 28 days on diets ranging from 100 to 1800 ppm 2,4,5-T with 0.5 ppm TCDD, retained substantial amounts of the dioxin. At a TCDD exposure level of 50 ppt (100 ppm 2,4,5-T) a level of 103 ppt was observed in fatty tissue. At a 900 ppt TCDD exposure, this level increased to 1120 ppt. Uptake of TCDD from the diet averaged 23% over this range of exposure levels.

Dr. Logan Norris of the U.S. Forest Service, Corvallis, Oregon described the collection of fish and wildlife samples from forested areas having previous histories of 2,4,5-T treatment for pine release. Areas selected for monitoring were all from the Pacific Northwest and had received relatively high rates of treatment (broadcast application) during July and August of 1973 at rates of three pounds active ingredient per acre. Ten different areas were sampled. At EPA's request, initial samples from these areas were to include: carnivorous, herbivorous and omnivorous fish, birds and mammals. However, in actual practice, it proved difficult to collect adequate numbers of certain subgroups e.g., herbivorous fish and carnivorous birds. Any future monitoring should take these difficulties into consideration. Also, careful pre-planning is necessary for timely and efficient collection of samples which at best are costly and require high manpower comitments.

Dr. Getzendaner of Dow Chemical Company briefly described completed monitoring studies for environmental residues of TCDD. These included

sampling of fish, water and sediment samples from both rice growing and rangeland areas. No positive TCDD residues were encountered for any of the samples collected in their program. Other relevant studies described by Dow included results of tissue analyses from a controlled feeding study wherein 2,4,5-T at a dietary level of 300 ppm was fed to sheep and the rate of decline measured after removal from treated feed. For fatty tissue, the t 1/2 for TCDD elimination was determined to be about 6 weeks. Other tissues gave results with different absolute levels but similar slopes. Also, a dairy cow feeding study was described wherein cows were fed dietary intakes of 2,4,5-T at about 500 parts per trillion containing 0.5 ppm TCDD. After 3 weeks exposure, the cows were placed on dioxin free diet and milk samples taken over a 90 day period. A t 1/2 for dioxin elimination of about 6 weeks was established from this study.

Captain Alvin L. Young, U.S. Air Force Academy described monitoring efforts conducted at Eglin A.F.B. Florida on a one square mile test grid over a period of 6 years. During this period, ecological effects on both plant and animal communities were observed. In addition to measurements on the grid area itself, forested areas and streams immediately adjacent to the grid were also observed. Chemical analyses for TCDD on liver and fat tissue from rodents collected in the treatment area were also discussed. A complete description of completed monitoring studies is included in Technical Report AFATL-TR-74-12, Unclassified, U.S. Air Force, Eglin AFB., Florida.

SESSION IV and V, WORKSHOPS

Analytical Methodology

Toxicology

Monitoring and Residues

I. ANALYTICAL METHODOLOGY WORKSHOP, July 26, 1974

A. Cleanup (Methodology)

1. The Baughman-Meselson procedure or modifications thereof are acceptable. There are certain basic essentials which must be included when using this method. Two separation steps are absolutely essential:

   a. Column chromatography step such as alumina, Florisil, silica gel, etc.

   b. Gas liquid chromatography step.

      (1) Important factors when using GLC:

         (a) Time of collection of eluent is very important. Time element beyond 30 seconds etc. impurities begin to elute i.e., impurities other than PBC's

         (b) Time factor is also important when using GLC-MS.

      (2) Important factors which should be taken care of in-house

         (a) Standardization of column packings both for column chromatography and gas chromatography.

         (b) In-house check on recoveries particularly for those laboratories which do only cleanup and where the samples are to be shipped to another laboratory involved in quantitating the dioxin content.

2. Other possible cleanup procedures which are currently being investigated and show promise for adaption to dioxin analysis:

   a. Neutral extraction technique

      (1) FDA

      (2) Harvard University

      (3) EPA (Mississippi Test facility)

   b. Gel permeation technique (Dr. David Stallings)

B. Instrumentation

1. Present available and functional systems

   a. Gas liquid chromatography-mass spectrometry

      (1) Low resolution

         (a) This technique is adequate for routine analysis. When interferences are incurred it would be necessary to go to high resolution mass spectrometry. The types of interferences with the two types of instruments are not the same. Therefore, both types of instrumentation should be available.

      (2) High resolution (discussion, same as above).

      (3) Medium resolution (There is no special advantage of having this particular technique available. However, its resolution is a somewhat better than low resolution but for dioxin analysis the added resolution affords no enhancement for TCDD analysis.

b. Chemical Ionization High Resolution mass spectrometry

This technique will be used only to complement the electron impact method. Due to the underlying principles of the method, the advantages of the technique are as follows:

(1) Sensitivity is comparable to the electron impact MS.

(2) Specificity is enhanced for dioxins in the presence of interferences such as PCD's and DDE, etc.

c. Other mass spectrometric techniques which are in their infancy and show some potential for dioxin analysis.

(1) Atmospheric Ionization (Dr. Vander velde, National Center for Toxicological Research, Pine Bluff, Arkansas).

(2) Plasma Chromatography (Dow Chemical Company, Midland Michigan).

(3) Radio-Immunoassay Technique (Dr. Jim McKinney, National Environmental Research Center, Research Triangle Park, North Carolina).

(4) Electron Spin Resonance Hyperfine Labeling Technique (Dr. Barry Commoner, Washington University, St. Louis, Mo., and Dr. George Yang, Food and Drug Administration).

d. Enzyme Induction Biosassay Technique. Suggested aryl hydrocarbon hydroxylase as a possible screening technique (Dr. David Firestone, FDA, Washington).

e. Round Robin Survey

EPA and FDA suggested that the two agencies jointly look into the possibility of obtaining a toxic reference standard to submit to all research institutes involved in dioxin analysis. This would enable some assessment to be made on the reliability of each other's data.

f. Interlaboratory collaboration. Everyone who participated agreed that interlaboratory collaboration should be made. This would include:

(1) Government

(2) University

(3) Industry

Participants in Analytical Methodology Workshop

Dr. Warren Crummett
Dow Chemical Company

Dr. Lewis Shadoff
Dow Chemical Company

Dr. Rudy Stehl
Dow Chemical Company

Dr. David Firestone
FDA, Washington, D.C.

Dr. George Yang
FDA, Washington, D.C.

Dr. Jack Plimmer
USDA, Beltsville, Md.

Dr. Aubry Dupuy
EPA, Mississippi Test Facility

Mr. Coleman Hall
EPA, Beltsville, Md.

Dr. Ed Oswald
EPA, Research Triangle Park, N.C.

Dr. Ralph T. Ross
EPA, Washington, D.C.

Mr. Peter Tarassoff
EPA, Dr. Upholt's Office, Washington, D.C.

Dr. Gunter Zweig
EPA, Washington, D.C.

Dr. Jim Olin
NCI, Tracor-Vitco, Rockville, Md.

Dr. Anthony Vithayathil
Washington University, St. Louis, Mo.

Dr. Mason Hughes
Aerospace Laboratories, Ohio

Dr. Leo Rodrigues
Aerospace Laboratories, Texas

II. Toxicology Workshop, July 26, 1974

Dr. Leonard Axelrod stated that any implementation plan on the toxicity of dioxins must first consider the following priorities:

1. Is dioxin, especially TCDD, in the environment?
2. How much is there?
3. What are the effects in the environment?
4. In laboratory toxicity studies, what levels show effects?
5. How do the environmental levels relate to those doses showing adverse effects in toxicological experiments?
6. In what areas are toxicity data needed?

The discussion then centered on adverse effects of subacute doses of TCDD as evidenced by the Dow 13 week study and the EPA 28 week study, both in rats. Drs. Axelrod, Colucci, and Gehring tried to resolve what would be the most sensitive indicator of TCDD exposure. Porphyria was emphasized since it was noted in both EPA's and Dow's subacute experiments and can also be found in humans exposed to certain drugs and pesticides. Dr. Colucci thought that various liver enzymes would be the most sensitive indicators of TCDD exposure whereas Dr. Gehring believe that pathological alterations of the liver or other tissues would be more sensitive. He cited TCDD's adverse effects in Dow's 13 week subacute intubation study, e.g., at 0.1 ug/kg/day 5 times/week there were increased S.E.R., some vacuolization in the liver and increased porphyrin excretion in the females; at 0.01

ug/kg/day the experimental animals were similar to controls. This can be compared to teratogenic studies at Dow in the rat in which embryotoxic effects were noted at 0.125 ug/kg/day during the critical phases of pregnancy, but not at 0.03 ug/kg/day.

Dr. Gehring then stated that Dow was planning a combination two year feeding study and a three generation study in rats to begin this fall. Dosing levels may be 0.1, 0.01, 0.001 and 0 ug/kg/day, 5 times a week based on Dow's 13 week study. Other rats would be exposed to TCDD 90 days prior to mating. Cytogenic screening would also be included. Similar long term and reproduction experiments are planned using 2,4,5-T.

Dr. Shibko thought that someone should test the teratogenic potential of TCDD on the golden hamster, whereas others,including Mr. Burnam and Dr. Holson, thought that complete studies should be done first in the mouse since previous studies did not indicate a no adverse embryotoxic or teratogenic level. It was learned that Dow was planning to soon begin such studies in the CF-1 mouse. N.C.T.R. was also planning extensive teratology study using TCDD in two strains of mice. This would be combined with a pharmacodynamic study in mice. The N.C.T.R. work would not begin until 1976.

A discussion of the pros and cons of a non-human primate teratogenic and reproduction followed. It was the opinion of Drs. Axelrod and Upholt that since there were no data on the effects of TCDD on monkeys that such a study would be worth the cost and should be done. The general idea of a one generation reproduction study and a teratogenic study was favored by all present. A consensus protocol is as follows:

1. Prior to mating, male and female non-human primates (in order of preference baboons, then Rhesus monkeys and Cynomologous) will be intubated with 2,3,7,8-TCDD for approximately 90 days. Some of the offsprings will be exposed to TCDD via mother's milk thoughout weaning. Other observations will be carried out in a manner consistent with a one generation reproduction study. Some mothers would be sacrificed and examined to observe the subacute effects of TCDD on non-human primates. It is possible that some pharmacodynamic data may be obtained.

2. The second major aspect of the non-human primate study would involve a teratology study whereby TCDD would be given in various regimens during pregnancy. There should be a minimum of 2 such dosing periods:

    a. from day 1 throughout the entire pregnancy, approximately day 162-165.

    b. during the critical period of organogenesis, day 20 through 43.

In both the reproduction and the teratology study, data from Dow's and EPA's subacute study in rats would be used to select doses. Three such doses would possibly be 0.1, .01 and .001 ug/kg/day 5 times/week. Numbers of animals and other paramaters would be decided later but it was thought that more animals should be used at the lowest dose in order to obtain a more meaningful statistical measurement.

Dr. Calvin Menzie, USDI, thought that fish and wildlife toxicity studies should be carried out with TCDD, but USDI did not have facilities to work with TCDD. He planned to send EPA his ideas of research needed in the fish and wildlife areas. Representatives from the Air Force indicated that they were interested in studying the effects of TCDD to any wildlife exposed during 2,4,5-T spraying. They were also to observe if TCDD could be ingested during grooming.

Monitoring of high risk populations was discussed but no consensus was reached. It was agreed that this was only the first of many regular meetings in this area.

Toxicology Workshop Attendees

Leonard R. Axelrod
EPA, Washington, D.C.

William Burnam
EPA, Washington, D.C.

Anthony Colucci
EPA, RTP, North Carolina

William Upholt
EPA, Washington, D.C.

Joseph Holson
NCTR, Jefferson, Ark.

John Young
NCTR, Jefferson, Ark.

S. I. Shibko
FDA, Washington, D.C.

Calvin Menzie
USDI, Washington, D.C.

B. Mason Hughes
USAF, Wright-Patterson, AFB, Ohio

Colin Park
Dow Chemical Company
Midland, Michigan

Perry Gehring
Dow Chemical Company
Midland, Michigan

III. MONITORING AND RESIDUES WORKSHOP, July 26, 1974

Dr. Getzendaner of Dow Chemical described an ongoing residue profile study wherein three beef cattle are fed on a diet containing 300 ppm 2,4,5-T containing 0.1 ppm or less of TCDD for a period of 20 days. Omental fat will be collected for a 24 week period at intervals of 0, 2, 3, 12, 16, 20 and 24 weeks. Tail fat will also be sampled on the 12th and 24th week. Study is simulative of the highest possible exposure situation which might be expected for rangeland treatment conditions under current patterns of accepted usage. With the GLC-MS procedure used by the Dow Chemical Company, any TCDD found is stipulated by their analysts to be 2,3,7,8 tetrachloro dibenzo-p-dioxin and not one or more of the other possible isomers, Other studies either planned for the near future or already underway include:

1. A surveillance of six or seven areas in which 2,4,5-T is known to be used on pasture and/or rangeland and where local markets can be found in which the beef produced in the surrounding area are marketed.
2. Collection of milk from markets in areas where 2,4,5-T use can be documented and where local markets can be found in which the milk produced in the surrounding area is marketed.
3. Collection of rice samples from specific areas treated last year with 2,4,5-T.

This project is being done in conjunction with the Mississippi Rice Growers Association. "Control" samples will also be collected. Adequate methodology for analysis is already available. In addition to this sampling program, Dow has requested all of their sales offices within the U.S. to purchase local off-the-shelf samples of rice from local supermarkets.

It was generally agreed that analyses of whole rice grain (unpolished) and rice straw were desirable during the initial phases of a monitoring program. Then, if residues are found additional samples aimed at isolating the exact location(s) of the residues in the product can be taken.

Mr. Crockett of TSD described the EPA rice monitoring program wherein 30 rice growing sites in Arkansas and Mississippi have been sampled. All of these sites have received recent treatments with 2,4,5-T.

Both Dow and EPA briefly described their catfish monitoring programs. Dr. Sterling suggested that other species, perhaps crayfish, also should be monitored. After considerable discussion, it was agreed that any future monitoring program should include additional aquatic species other than catfish and crops rotated with rice. Soybeans are a preferred sample since they have high oil contents and would most likely pick up more dioxin than the less oily crops, if, indeed, translocation does occur.

Captain Alvin Young, U.S. Air Force also described translocation studies underway at Eglin A.F.B., Florida. Sorghum plants grown in soil containing 250 ppt of TCDD will be sampled for seeds, leaves, and roots. TCDD analyses at parts per trillion sensitivities will be performed on these samples under contract with the Dow Chemical Company. Also, beans rotated with the sorghum will be sampled and analyzed for TCDD.

Captain Young also described ongoing food chain monitoring for dioxins in an aquatic area receiving runoff or eroded soil from a one square mile test grid located at Eglin A.F.B. Dioxin analyses of the bottom sediments in this area showed levels of about 13 parts per trillion. A number of aquatic species ranging from insect larvae to large fish will be analysed.

Dr. Sterling then brought up the use of silvex in Canada for pre-harvest fruit drop control and indicated that there is considerable concern in certain quarters over dioxin residues which might obtain from this use. On further discussion it was established that extremely dilute (ca. 20 ppm) formulations are used and that the total potential residues on apples would be far less than the sensitivity of the current analytical methodology. Since the meeting, calculations have been rechecked and maximum possible residues calculated in the range of 10^{-14}g TCDD/g apple.

Mr. Crockett of EPA described an ongoing residue program wherein 200 acres of Texas rangeland have been treated with 2,4,5-T at a rate of 1/2 pound/acre. Sheep were placed on the treated range immediately after spraying, grazed for a period of one month and then placed on untreated range for a two week period. This test is a cooperative effort with the Texas Agricultural Extension Service. In addition to sheep, wild jack rabbits and deer will be collected both prior to and after treatment with 2,4,5-T. The advisability of conducting additional wildlife monitoring programs was discussed but the general consensus was to delay, pending routine confirmation of EPA/s tentative analytical data.

Captain Young stated that programs are now underway to establish the route of entry of dioxins into rodent species resident in the Eglin test grid area. Dietary intake of these animals is about 90 percent seed and 10 percent insects. Analysis of typical seed samples from the same area show no detectable TCDD at part per trillion sensitivity. It is speculated that the dioxin found in these rodents is the result of a transfer from the soil surrounding their burrows. This may take place in the grooming process.

Further analyses of rodent skin and other body parts will be made in an effort to establish the route of entry. In any event, results of these route of entry studies may be helpful in the planning of future monitoring tests on rights-of-way and in forests.

Dr. Logan Norris suggested that analyses of water and suspended sediment may be very important. He stated that 75 percent of the nation's water originate in forested areas. To achieve maximum monitoring sensitivity, any sample collections should be made in forest streams where dilution effects would be minimal.

Dr. Kutz discussed EPA's human monitoring program wherein human adipose, liver and milk samples have been collected in States having high 2,4,5-T usage for weed control in rice culture. These samples, collected in Mississippi and Arkansas, have not yet been analyzed and are awaiting development of a suitable analytical procedure.

Dr. Sterling suggested that placental tissue might be a valuable additional source for human sampling and that good case histories are readily available. There was some discussion with regard to the legal constraints imposed on human tissue sampling. Informed consent is mandatory and this situation applies to all types of tissues, even from deceased individuals, where next of kin must be notified. Dr. Sterling also suggested sampling of young couples living in wilderness areas adjacent to various utility right-of-ways. Presumably, these people may be directly exposed to spray drift from herbicide applications.

The need for selective sampling at the outset of any expanded monitoring program was emphasized. Otherwise, residues of significance may be missed in a randomized, non-directive sampling program. Initial efforts should be aimed at sampling of high exposure groups. If these groups do not show demonstrable levels of dioxin, additional monitoring may be unnecessary. Dr. Klingman suggested that an excellent source of high exposure human samples would be ground foliage spray crewmen conducting treatments on right-of-ways. Dr. Kutz indicated that a number of individuals having a documentable high exposure to dioxin containing products have been recruited as tissue (adipose) donors but that actual arrangements for surgical biopsies will not be made until a sensitive analytical procedure is available. Several of these individuals have active cases of chlorance. The evaluation and end use of residue analyses was also discussed. It was generally agreed that the level of incidence as well as the toxicological significance of any particular level of the residue must be carefully considered.

The potential formation of dioxins due to pyrolysis from burning of 2,4,5-T treated forest areas was also discussed. Mr. Collier described EPA laboratory studies whereby sodium trichlorophenate and/or the sodium salt of 2,4,5-T were converted to TCDD at a yield of 0.15 to 0.3 percent. These data confirmed earlier studies by Baughman at Harvard and at Dow where similar levels of TCDD formation were reported. Higher levels, as reported by Buu-Hoi, could not be demonstrated by any of the three other groups. To date, it has not been possible to duplicate this dioxin formation in the laboratory by burning of 2,4,5-T laden organic matter. However, studies have been of a very simple design and not simulative or real forest conditions. It was

brought out by Dr. Martin that several facilities within the U.S. Forest Service would have the necessary equipment to conduct more realistic burning studies. It was generally agreed that additional studies would be desirable but that studies under actual field conditions should not be undertaken. In the Western U.S., burning is done 2-4 months after application of herbicide whereas in the southern part of the nation, 3-5 months are allowed to elapse before burning.

Mr. Collier discussed tentative EPA plans for future residue/monitoring programs. These included:

1. Survey of Use Patterns Associated with the Pesticides Erbon, Silvex, Ronnel and 2,4,5-trichlorophenol.
2. Establishment of "Indexes of Exposure" for Selected Human Populations.
3. Monitoring of Fish Downstream from Cooling Towers.
4. Sample Aquatic Sites Treated with Silvex.
5. Monitoring of Aquatic Organisms from Treated Rice Growing Areas
6. Additional Monitoring of Forests and Right-of-ways.
7. Monitoring of Human Adipose or Mother's Milk from High Risk Exposure Groups.
8. Sampling of TCDD in Fat from Domestic Ruminants Killed in Slaughterhouses.
9. Dairy Cows Study - TCDD Transfer to Cow's Milk.

Finally, the need for additional sampling of domestic ruminants from rangeland was discussed. Since controlled laboratory studies on beef cattle are now underway, it was deemed appropriate to delay additional sampling pending outcome of the residue uptake decline studies. Dr. Klingman suggested that future studies consider sampling of local grass fattened animals where the logistics of surveillance could be georgraphically restricted.

Monitoring Workshop Attendees

C. W. Collier
EPA, Washington, D.C.

Alan Crockett
EPA, Washington, D.C.

Rick Kutz
EPA, TSD, OPP, Washington, D.C.

J. H. Davidson
Dow-Midland, Michigan

M. E. Getzendaner
Dow-Midland, Michigan

Jim Martin
National Forest Products Assn.,
Butler, Alabama

Roger E. Sandquist
Forest Service, Washington, D.C.

Logan Norris
Forest Service, Corvallis, Ore.

Mike Taylor
U.S. Air Force, Wright-Patterson AFB

Ted Sterling
Simon Fraser University, Canada

Al Young
USAF Academy, Colorado

Margaret Brienholt
USDA, Office of General Counsel

Dayton Klingman
USDA, ARS (Rep. Weed Science Society,
Beltsville, Maryland

Roy Johnson
Amchem, Ambler, Pennsylvania

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Criteria and Evaluation Division

August 30, 1974

Dr. Alvin L. Young
U.S. Air Force
U.S. Air Force Academy,
Colorado 80840

Dear Dr. Young:

Enclosed please find a summary of the Dioxin's Conference and workshops held recently at the Mayflower Hotel in Washington, D.C.

I would like to personally thank you for your attendance and valuable contribution at this Conference and look forward to your continued interest and cooperation.

Sincerely,

Leonard R. Axelrod

Leonard R. Axelrod
Director

PRELIMINARY DIOXIN

IMPLEMENTATION PLAN

U.S. ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF PESTICIDE PROGRAMS

CRITERIA AND EVALUATION DIVISION

WASHINGTON, D.C. 20460

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I. INTRODUCTION

A group of homologous compounds known collectively as chlorodioxins (dioxins) are produced as side products in the technical materials of a number of known registered pesticides. One of these dioxins, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is known to have high acute toxicity to mammalian species. In fact, this compound is at present the most toxic small molecule known to man, e.g., in the guinea pig its toxicity is approximately 5,000 times more than that of the most toxic organophosphate pesticide. Furthermore, TCDD has been shown to be a potent teratogen, and its presence may exert delayed and/or cumulative toxic effects.

Concern over possible teratogenic effects of the herbicide 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) which contains TCDD as an impurity led to the cancellation of this product for use around water, homes, and food production. Due to the absence of formal appeals by affected registrants, all of these uses with exception of its use on rice were deregistered.

Appeals sought by registrants for continuation of the currently registered use of 2,4,5-T on rice led to scheduling of a public hearing. In its final form, the hearing was to represent a consolidation of the registrants' appeals for the use of 2,4,5-T on rice and EPA's fact finding (6-b-2 type) hearing for all other previously registered uses.

As part of EPA's preparation for a public hearing, a monitoring program for TCDD in human tissue, human mother's milk, fish and wildlife within the continental U.S.A. was initiated. The entire

program depended largely on the reported availability of a newly developed analytical method employing highly sophisticated instrumental techniques for quantitating TCDD at the parts per trillion (ppt) levels.

In March, 1974 it became apparent that unanticipated problems had arisen with the recommended analytical method which could not be solved by the time of the originally scheduled hearings to begin in July 1974. For this reason, the Acting Administrator of the EPA announced the cancellation of the July - hearings but that the Agency would continue its TCDD residue monitoring program and take further action once the results of the monitoring project become available.

A modification of a method developed by Baughman and Meselson was selected for EPA sponsored monitoring programs. [R. Baughman and M. Meselson, Environmental Health Perspectives, September, (1973)]. As originally described. this technique employed cleanup with preparative GLC-cleanup and alumina adsorption chromatography followed by direct probe insertion high resolution mass spectrometry (MS) coupled to a time-averaging computer (CAT). TCDD has been analyzed by this method from samples of human mother's milk, fish and shellfish received from South Vietnam.

The modified method recommended by Baughman for use by EPA eliminated the GLC preparative step and added a second adsorption chromatography step. This modification was proposed by Baughman as a direct substitute for the original method. According to Baughman, this method would not only give more reproducible recoveries of TCDD but would also afford adequate sensitivity and specificity for samples of U.S. origin, including minimization of interferences due to polychlorinated biphenyls (PCB's) and p,p'-dichlorodiphenyl-2,2-dichloroethane (DDE).

Recently, however, some questions have been raised on the reliability of data obtained by the modified method which are as follows:

1. The current method for quantitating TCDD is by peak ratio, m/e 320, m/e 322. These are the two most intense peaks in the isotope cluster of the molecular ion of TCDD. Both DDE and PCB's which may be impurities in the sample substrate interfere in this region of the mass spectrum. Therefore, there has been some concern over the data as being a true representation of TCDD levels. These interferences have been observed by Baughman, Dow Chemical and EPA. The interferences are found more frequently in human mother's milk and adipose tissue than in other substrates. Regardless of the number of samples where the interferences have blotted out the TCDD area in the mass spectrum, there have been many samples which have ranged from "minutely suggestive" to "highly suggestive" for the presence of TCDD.

2. Recently, Baughman reported an impurity of higher molecular weight, m/e 446 in Vietnamese samples. This compound could conceivably fragment, and one of its fragment ions may have the same m/e as TCDD which would contribute to higher TCDD values.

   Dow Chemical Company reported an interference with similar characteristics from Arkansas catfish samples. These workers have eliminated the problem by developing a procedure utilizing gas liquid chromatography interfaced with mass spectrometer (GLC-MS). To date, the Pesticides and Toxic Substances Effects Laboratory (PTSEL) has not observed this interference.

3. PTSEL has recently reported other interferences which have been attributed to sample handling. However, PTSEL has in part resolved this problem.

A recent meeting was held by EPA with members of PTSEL, C & E, R & D and OGC to make an assessment on the TCDD data obtained so far. The conclusions are outlined in the following:

1. The modified Baughman method has proven definitive only for shrew samples. Due to the number of interferences, data for TCDD analyses of other samples are only suggestive for the presence and quantity of TCDD.

2. More interlaboratory collaboration is needed for data comparison.

3. Other techniques such as high resolution chemical ionization mass spectrometry (CI-MS) and gas liquid chromatography mass spectrometry (GLC-MS) should be utilized. A combination of these techniques will offer the sensitivity and specificity for more reliable identification of TCDD.

Recently, a meeting was held by EPA to evaluate the current status of the various dioxin programs and to plan future objectives. Representatives from government, industry and academia participated in this meeting which was divided among three important areas - analytical methodology, toxicology and monitoring. A summary of this meeting is given in the attachment titled Summary of Dioxin Planning Conference, Mayflower Hotel, Washington, D.C., July 25 - 26, 1974.

As a result of this meeting, the following programs related to analytical methodology, surveys of use patterns, toxicology and estimates of amounts of human exposure and environmental monitoring are proposed. Certain of these proposals are of necessity dependent on the completion

of others, and, therefore, a multi-element program encompassing three fiscal years, as outlined in Figure I, is proposed. Individual elements of the program are detailed in the next section.

II. SCOPE OF WORK

A. Analytical Methodology for Environmental Samples

As previously noted in the Introduction, the modified Baughman-Meselson procedure is not generally applicable to all matrices and has been found to be inadequate due to known and unknown interferences. The modified method involves adsorption multiple column chromatography using alumina followed by direct probe high-resolution mass spectrometry.

Baughman-Meselson were successful in analyzing biological samples for ppt TCDD using the original, published method [Environmental Health Perspectives, September 1973]. This method utilizes preparative gas liquid chromatography (GLC) prior to mass spectral analysis. On the other hand the unpublished modified procedure recommended to EPA by Baughman omits the GLC step, and has been shown to be inadequate for absolute quantitation of TCDD by direct probe electron impact mass spectrometry due to the inability of absorption chromatography to separate some impurities present in the samples substrates. Dow Chemical Company has demonstrated the importance of the GLC step and their success in analyzing TCDD by interfacing the gas liquid chromatograph to a low resolution mass spectrometer (GLC-MS). Due to the number of interferences encountered by the modified Baughman technique utilizing direct probe insertion high resolution mass spectrometry, EPA has re-evaluated their current methodology and is revising the analytical methodology for this project to other highly sensitive and specific analytical instrumentation methods. The cleanup procedure available and methodology to be developed in for this effort are described in the following:

1. Cleanup Procedures

Existing cleanup procedures or modifications thereof are adequate for dioxin analysis. These are basic essentials which are required in cleanup methodology.

a. Adsorption chromatography, alumina, Florisil, silica gel.

b. Standardization of column packings.

c. In-house monitoring of recoveries, particularly those laboratories involved in cleanup of samples to be shipped to other facilities for further analysis.

2. Gas Liquid Chromatography Mass Spectrometry (GLC-MS)

a. GLC Low Resolution-MS - Low resolution (Resolution 400) GLC-MS has been demonstrated by Dow Chemical Company to be an adequate technique for routine monitoring of dioxins. The sensitivity depends on sample type, and for some samples sensitivity as low as 0.5 ppt has been achieved. When interferences are encountered, high resolution GLC-MS should be available since the types of interferences found with the two types of GLC-MS are not necessarily the same.

b. GLC High Resolution-MS - There is no special advantage of using high resolution GLC-MS routinely for dioxin analysis. However, it is desirable to have this technique available to resolve interference problems found with low resolution GLC-MS. Also, the types of interferences encountered with high resolution GLC-MS are different from those found in low resolution GLC-MS as mentioned above. The major advantage of high resolution GLC-MS technique is its resolving power to differentiate among ions of closely related mass.

3. High Resolution Chemical Ionization Mass Spectrometry (CI-MS)

High resolution chemical ionization both positive (CI) and negative (NCI) mass spectroscopy offers numerous advantages for pesticide analysis as compared to EI. The primary advantages stem from the relative simplicity of CI and NCI spectra, their comparable sensitivity to EI, the fact that both CI and NCI spectra are dominated by ions of high information content that are close in mass to the molecular ion (molecular ions in EI spectra of pesticides often have low intensity). The combined use of CI and NCI spectra may afford an additional advantage of improved reliability of the analytical procedure, and simplify the identification of a given residue. Utilization of this procedure will also reduce the requirements on "clean up" and thereby improve recovery for unstable compounds. A further advantage is that once a validated high resolution CI method is available, there exists the possibility for development of low resolution, less expensive mass spectrometric procedures of environmental samples and pesticide formulations.

4. Other Techniques showing Potential for Dioxin Analysis

a. Atmospheric Ionization Mass Spectrometry

Picogram quantities of compounds are introduced into the ^{63}Ni source of the mass spectrometer and the sample is ionized at atmospheric pressure. The underlying principles of the

technique are quite similar to those of chemical ionization mass spectrometry. The technique is sensitive and will afford complementary data to the eletron impact as well as chemical ionization mass spectrometry.

b. Plasma Chromatography

The technique is very sensitive, but, to-date, there are instrumental problems which have not been totally resolved. Dow Chemical Company is currently investigating its potential for dioxin analysis.

c. Electron Spin Resonance Technique (ESR)

This technique will afford differentiation among the various chlorinated dioxin isomers. However, there are certain limitations to the method and, to-date, these have not been fully resolved. Spectra of the pure sample compounds of the various chlorinated dioxins must be available for comparison, and as the dioxin project progresses, other dioxin isomers may become available. The sensitivity of the technique diminishes at the picogram level, but as soon as Fourier Transform ESR is available, the sensitivity factor should be enhanced considerably.

d. Radio-Immunoassay Techniques

This method is potentially capable of extreme sensitivity at sub-picogram (parts per quadrillion) levels. Sample cleanup would be minimal and analytical output could be many samples per week (vs. about ten for GLC-MS). This method would be well suited to routine surveillance programs. The potential for high specificity of this method with regard to individual dioxins and the lack of homologous series interferences cannot be predicted in advance and can only be determined after completion of a pilot study program.

e. Enzyme Induction Bioassay Technique

The method will afford a technique to scan a sample quickly in order to check for the presence of dioxins. The method is sensitive, and a large number of samples may be screened over a short time period. A suggested enzyme is an aryl hydrocarbon hydroxylase. (Also, See Section B.1.)

B. Analysis of Technical Grade Pesticides and/or Their Formulations for TCDD in Parts Per Billion (ppb)

Once one of the instrumental or biochemical methods described above have been developed, it is anticipated that certain modifications may be made in order that analysis of formulations may be

conducted in field laboratories. Currently used methodology is sensitive only to the 100 ppb level which appears inadequate sensitivity for this survey. A suggested technique is low resolution mass spectrometry combined with gas-liquid chromatography. This particular technique will depend on completion of part A.

An alternate screening procedure for TCDD in Technical Grade Pesticides and/or their formulations may be the enzyme induction bioassay, described above (See II. A 4 e.)

C. Toxicology

There has been a large amount of recent information generated on the toxicity of the dioxins and specifically on 2,3,7,8 TCDD. Such current and planned projects should be available to interested researchers in order to avoid duplication in costly experimentation with the toxic dioxins and to provide feedback in areas of protocols, species and dosages. This section of the implementation plan is designed to reveal current and proposed research plans of governmental agencies, universities and industries with the various dioxins and to discuss needed toxicological research that is not yet firmly delineated.

1. Currently Funded or Planned Studies.

a. Subacute Studies

(1) EPA is sponsoring a 28 week subacute intubation study in male and female Sprague-Dawley rats at IITRI, Chicago, Ill. with a 12-week withdrawal period. In addition to histopathological examination of various organs, TCDD-residue analysis will be carried out on the same organs once the method becomes available. In is hoped, that the onset or disappearance of pathological changes in various tissues can be related to TCDD levels in the same tissues. Doses of TCDD used were 1.0, 0.1 and 0 ug/kg week which approximated 3.0, 0.3 and zero TCDD in the rat's daily diet.

(2) Dow Chemical Company has recently completed a 13-week subacute intubation study combined with a 7-week pharmacodynamic study in rats. In addition to the controls, the rats were dosed with 1.0, 0.1, 0.01 and 0.001 ug/kg/day TCDD five times a week. Besides histopathological examinations, any changes in porphyrin excretion and serum enzymes were checked.

(3) IITRI, Chicago, Ill., under contract to National Cancer Institute, is in the process of testing various dioxins for carcinogenic activity. Prior to long term studies, most dioxins will undergo a 13-week subacute study to determine a maximum tolerated dose. IITRI has almost completed a 13-week study with TCDD, and later will begin similar studies with the hexachlorodioxins.

b. Long-term exposure (Tumorigenic)

(1) Dow is planning a two-year study with TCDD in rats beginning this fall. In tandem with this, a three generation study in rats is planned. Doses obtained from Dow's 13-week subacute experiment will be the basis for the dosing. Similar work in both areas is planned for 2,4,5-T.

(2) Current tumorigenic studies at IITRI, under the direction of Tracor Jitco, managing various contracts for the National Cancer Institute, is on the following schedule.

(a) Unsubstituted dioxin - Completed all aspects.

(b) 2,7, dichlorodioxin - Completed skin painting and cancer promotion study; oral to rats and mice almost finished.

(c) trichlorodioxin - Contains 2,3,7,8 TCDD; currently working of obtaining a pure product.

(d) 2,3,7,8 TCDD - Almost finished a 90-day subacute intubation range-finding study for oral studies; have determined a maximum tolerated dose for skin painting in mice. Carcinogenic data with TCDD will probably not be available until 1977.

(e) Hexachlorodioxin - Work in planned with 1,2,3, 6,7,8 and 1,2,3,7,8,9 isomers .

(f) Octacholorodioxin - Increased mortality due to contamination by hexachlorodioxins has put this project off schedule. Work will begin using octachlorodioxin having no hexachlorodioxin impurities.

c. Teratology

(1) Dr. Tom Collins of FDA Washington, D.C. is continuing his teratology work on various purified dioxins.

(2) National Center for Toxicological Research is planning teratology studies using large numbers of mice exposed to TCDD by both gavage and in feed. Doses will be less than 3 ug/kg/day, but this study will not begin until early 1976.

(3) Dow plans to begin in the near future a teratology dose-response study in the CF-1 mouse.

d. Mutagenicity

(1) Dr. Bruce Ames, University of California, Berkeley, California, plans to continue mutagenic studies with various mutant tester strains of S. Typhimurium. Previous studies at his laboratory have shown no mutagenic activity of TCDD.

(2) Dr. Sidney Green, FDA, Washington, D.C. is continuing in vivo cytogenic studies on the bone marrow of rats and guinea pigs.

e. Pharmacodynamic studies

(1) Subacute pharmacodynamic data has been mentioned in connection with EPA's and Dow's subacute work.

(2) Metabolism, distribution and excretion studies are planned by ORD, N.E.R.C. EPA in North Carolina and by N.I.E.H.S. using mainly TCDD.

(3) Dr. James Allen Univerity of Wisconsin Medical School, has recently finished a study involving the metabolism, distribution and excretion of a single dose of TCDD.

(4) National Center for Toxicological Research is planning a pharmacodynamic study of TCDD in mice and rabbits which will not begin until 1976.

f. Other studies

(1) ORD, NERC, EPA in North Carolina is looking at various aspects of TCDD subacute toxicity in rats obtained from EPA's contract at IITRI e.g., enzyme levels, porphyria, blood components.

(2) Dr. John Moore, N.I.E.H.S. is continuing work on the effects of TCDD on the cellular immune response and enzyme induction (Dr. George Lucier). Some general toxicity work with hexachlorodioxins is planned.

(3) USAF, DOD is carrying out environmental toxicology studies on organisms exposed to high levels of TCDD from 2,4,5-T applications.

(4) EPA, Gulf Breeze, Flordia is sponsoring an egg to egg chronic bioassay study on an estuarine minnow (Cyprinidon variegatus) involving low level exposure to TCDD. Exact levels will be extablished after preliminary toxicity ranging studies.

2. Proposed Studies

a. Proposed non-human primate reproduction and teratology study.

(1) Prior to mating, male and female non-human primates (in order of preference, baboons, then Rhesus monkey and Cynomologous) will be intubated with 2,3,7,8-TCDD for approximately 90 days. Some of the offspring will be exposed to TCDD via mother's milk throughout weaning. Other observations will be carried out in a manner consistent with one-generation reproduction study. Some mothers would be sacrificed and examined to observe the subacute effects of TCDD on non-human primates. It is possible that some pharmacodynamic data may be obtained.

(2) The second major aspect of the non-human primate study would involve a teratology study whereby TCDD would be given in various regimens during preganancy. There should be a minimum of 2 such dosing periods:

(a) from day 1 throughout the entire pregnancy (approximately day 162-165).

(b) during the critical period of organogenesis (day 20 through 43).

In both the reproduction and the teratology study, data from Dow's and EPA's subacute study in rats would be used to select doses. Three such doses would possibly be 0.1, 0.01 and 0.001 ug/kg/day 5 times/week.

b. Proposed fish and wildlife studies.

Dr. Calvin Menzie of USDI, Washington, D.C. has agreed to submit a protocol for fish and wildlife toxicity studies needed with TCDD. Dr. Logan Norris of USDA and scientists from USDI in addition to EPA's Criteria and Evaluation personnel could provide an input to needed research.

c. Subacute and reproduction studies on other dioxins.

NCI's long term studies with various dioxins usually are preceded by a 13-week range finding study in rodents in which a maximum tolerated dose in sought. Such data may provide adequate subacute information. No single or multi-generation studies are planned with dioxins.

D. Monitoring and Residue Studies

To-date, EPA sponsored monitoring programs on rights-of-ways, forests and rice growing areas have tentatively suggested the presence of TCDD at parts-per-trillion levels in certain fish and wildlife samples. With the exception of shrew samples from rights-of-ways, the analyses have not been confirmed and can only be considered as tentative. Final selection of any new expanded monitoring programs must of necessity first require development of an accepted analytical technique and the validation of the actual presence of TCDD residues in existing samples. Also, additional efforts in this area should not be initiated without considering the toxicological significance of the various levels or residues encountered and without the development of estimates of the expected frequency of occurrence of such residues. The following plan briefly outlines specific proposed studies for the future. The first two of these studies, namely - "Survey of Use Patterns Associated with the Pesticide Erbon, Silvex, Ronnel, and 2,4,5-Trichlorophenol" and "Establishment of Indexes of Exposure for Selected Human Populations" will bear heavily on any additional finalized residue/monitoring programs.

1. Studies in Progress

a. Dow Chemical Company

(1) A TCDD residue profile study is now in progress wherein three beef cattle have been fed on a diet containing 300 ppm 2,4,5-T containing 0.1 ppm or less of TCDD for a period of 20 days. Omental fat will be collected for a 24 week period at intervals of 0,2,3,12,16,20 and 24 weeks.

(2) Dow has initiated a surveillance of six or seven areas in which 2,4,5-T is known to be used on pasture and/or rangeland and where local markets can be found in which the beef produced in the surrounding area are marketed.

(3) Dow has initiated a collection of milk from markets in areas where 2,4,5-T use can be documented.

(4) A collection has been mode of rice samples from specific areas treated in 1973 with 2,4,5-T (In cooperation with Mississippi Rice Growers Association.

b. EPA Studies 1974

(1) A 2000-acre plot of Texas rangeland has been treated with 2,4,5-T at a rate of 1/2 pound/acre. Sheep were placed on the treated range immediately after spraying, grazed for a period of one month and then

placed on the treated range immediately after spraying, grazed for a period of one month and then placed on untreated range for a two week period. Residues in both sheep fat and liver will be analyzed. In addition to sheep, wild jack rabbits and deer will be collected both prior to and after treatment with 2,4,5-T. This test is a cooperative effort with the Texas Agricultural Extension Service. The 2,4,5-T formulation used in this test represented current production quality material wherein the TCDD content is 0.1 ppm or less.

(2) A fish residue study is underway whereby bluegill (Lepomis macrochirus) have been continually exposed in a flow-through system to TCDD at a constant level of 0.1 ng/liter. Fish samples were taken at 0,7,14,21 and 28 days. Similar samplings were made after transfer into dioxin-free water during withdrawal periods of 7 and 14 days. Analyses of these fish have not been performed.

c. U.S. Air Force, Eglin A.F.B., Florida

(1) An aquatic food chain monitoring study is now in progress wherein an aquatic area receiving runoff from a one-square mile herbicide test grid is being extensively sampled. Average TCDD content in the bottom sediments from this area is about 13 parts per trillion, as analyzed by Dow.

(2) Translocation studies involving sorghum plants and beans grown in TCDD laden soil (250 ppt) are now underway. Analyses have not yet been completed.

(3) The route of entry of TCDD into rodent populations indigenous to the Eglin AFB herbicide test grid is now under investigation. It has not yet been established whether the major cause of TCDD residues, ranging from 200 to 500 ppt in rats and mice, is due to oral intake or dermal exposure.

2. Proposed Studies

a. Survey of Use Patterns Associated with the Pesticides Erbon, Silvex, Ronnel and 2,4,5-Trichlorophenol

In addition to 2,4,5-T, the following pesticides are likely to contain detectable levels of TCDD; erbon, silvex, ronnel and 2,4,5-trichlorophenol. All of these are derived from technical 2,4,5-trichlorophenol as a starting material. A listing of all known patterns of use for these products is given in Table I.

For many of these patterns of use we have little knowledge of the extent of such use including amount, frequency or geographic distribution. Also, the degree and extent of pesticide applicator or bystander exposure through dermal, oral and inhalation routes is relatively obscure for many of these uses. Some of the uses listed in Table I., while at first glance appearing to be relatively innocuous, could possibly, on closer inspection, represent substantial environmental impacts ultimately reflected in man himself. For example, over four billion gallons of emulsified cutting oils were used in 1973. Little is known as to what portion of this total amount is associated with 2,4,5-trichlorophenol (Use Number 4, Table I.). Since the aqueous portion of these oils are ultimately discarded into the environment, any associated impurity such as TCDD having persistent and accumulative properties could represent serious potential problems to man by way of the aquatic environment. Another pattern of use which when first examined generates little or no concern is that involving 2,4,5-trichlorophenol as a textile finish preservative. A clower look at this pattern of use, however, suggests that in single industrial plants,

thousands of women of child bearing age may be exposed daily to low levels of the impurity TCDD, (a potent teratogen) for extended periods of time. This exposure may result not only from dermal contact with textile finish components on textile yarns but also from inhalation of textile finishing aerosols. Therefore, to adequately assess the potential impact on humans based on degree of exposure from various patterns of use will require a carefully considered survey. Special emphasis should be given to use pattern numbers 1 through 6 and 16 through 20 in Table I. Information obtained from the survey should include amounts used, geographic distribution of use, age and sex distribution of primary users, modes of application and relative amounts of various formulations used.

b. Establishment of "Indexes of Exposure" for Selected Human Populations

Based on results of the survey discussed above, and reliable information on the current dioxin levels in the various pesticide products, certain patterns of use could be singled out as particularly important in terms of degree, frequency and extent of human exposure to TCDD residues. Data from this survey could be used to estimate probable "indexes of exposure" from the various patterns of use.

After use of appropriate weight factors, relative degrees of impact on humans may be established. This in turn would lead to a listing of priorities for a sequence of monitoring operations. Residues of TCDD could be a direct result of dermal or inhalation exposure or indirectly via contaminated food. Those important use patterns showing the greatest probable degree of exposure to human food sources and/or humans themselves would be selected as first order candidates for an expanded monitoring program. After review of data from the more likely prospects, the program would be expanded stepwise to encompass all other patterns of human health significance.

c. Monitoring of Fish Downstream from Cooling Towers (See Table I. Use Pattern No. 6)

The bactericide 2,4,5-trichlorophenol is sometimes used as a cooling tower slimicide. Blowdown from these cooling towers is often discharged directly into rivers and streams. Depending on the particular system, this discharge may be continuous or intermittent. Due to the probable differential degradation rate of the trichlorophenol and TCDD in aquatic systems, it is likely that fish could accumulate TCDD even though not tainted with trichlorophenol producing an off-flavor.

It is proposed that several industrial and/or power plants housing cooling towers under treatment with trichlorophenol be monitored. Fish and molluscs will be collected downstream from the effluent discharge points and TCDD-residues compared to similar collections taken upstream from the plants.

d. Sampling Aquatic Organisms from Treated Rice Growing Areas

A preliminary monitoring program for TCDD in catfish collected from rice growing areas has resulted in tentative positive finds in two out of fifteen samples. All of these fish were collected in areas near or adjacent to rice fields having a known history of 2,4,5-T treatment. In some instances the fish were collected from sites receiving rice field irrigation floodwaters.

Additional sampling of rice treatment areas is highly desirable to further define the extent and magnitude of the residue problem. Since both 2,4,5-T and silvex are registered for use as rice herbicides, it is desirable to develop information on both of these pesticides. It is proposed that additional sites be monitored with equal distribution of sampling between areas treated with 2,4,5-T and silvex. In addition to catfish samples, other aquatic life including crayfish may be collected along with appropriate sediment samples.

e. Sample Aquatic Sites Treated with Silvex

The herbicide silvex is registered for control of submerged weeds in lakes and ponds. There are no label restrictions with regard to harvesting of fish and shellfish from these areas. Based on the persistent and accumulative characteristics of TCDD, there is concern over potential residues in fish and shellfish from these areas. Also, silvex is used by various federal agencies for aquatic weed control projects. Many of these patterns of use are not registered and entail use of silvex in streams and in coastal areas. Pollution of the estuarine environment with TCDD is a distinct possibility. To assess the significance of silvex for these varied aquatic uses, a sampling program involving about 100 samples divided equally between the major patterns of use is recommended. At least 5-10 different sampling sites should be selected with an equal distribution between fish (both top and bottom feeders) as well as shellfish included in the sampling. Sampling projects conducted in areas of federal use can probably be done cooperatively at little or not cost with the user agency conducting both the pesticide treatment and sample collecting programs.

f. Additional Monitoring of Forests and Rights of Ways

Any additional collection of samples from these areas will be dependent on the results of confirmatory analyses for TCDD from earlier monitoring programs. If positive TCDD analyses are confirmed, additional sampling will be needed to establish the routes of movement of dioxins through these ecosystems and to estimate the extent and degree of fish and wildlife exposure.

g. Monitoring of Human Adipose or Mother's Milk from High Risk Exposure Groups

If, on the basis of the use pattern survey (Table I.) and a careful assessment of the "index of exposure", any pattern of use listed in Table I is suggestive of excessive human exposure, sampling of human adipose tissue by surgical biopsy may be justified. A minimum of 20 samples should be considered for each exposure situation selected for monitoring. This program would be conducted by cooperating physicians within the EPA Community Studies Network. One pattern of use worthy of special mention at this time is that involving the potential exposure of women of child bearing age to house and garden herbicides which may contain TCDD. When the herbicide 2,4,5-T was cancelled for use around the home,

silvex was immediately substituted as a replacement. Since silvex may also contain TCDD, it too may cause human exposure to TCDD. If monitoring of this pattern of use is justified, based on estimated exposure indexes, a sampling program involving human mother's milk is proposed.

h. Sample TCDD in Fat from Domestic Ruminants Killed in Slaughterhouses

Attempts to collect samples of domestic ruminant tissues emanating from high 2,4,5-T use areas (treated rangeland) have been unsuccessful. Initial efforts were made cooperatively with the U.S. Department of Agriculture, but over an 8-month period only 6 samples were obtained. A second effort is desirable so that estimates of TCDD residues in meats reaching the consumer market can be made. This information in turn can be utilized in making recommendations to the Meat and Poultry Inspection Branch, Animal Plant Health Inspection Service, U.S. Department of Agriculture in terms of nationwide surveillance programs.

The program will require detailed record keeping and follow-up, including treatment history, dates of individual ruminant grazing on treated rangeland, history of shipment to feed-off lots and periods and dates of slaughter. If careful selections are made of rangeland treatment, grazing and feedoff conditions, it is estimated that 30-50 separate fat samples from each ruminant species will be adequate to define the residue problem, if any, and establish the need for larger scale programs conducted under the auspices of USDA or FDA. Initial phases of the program may involve sampling of grass fattened ruminants destined for local slaughter with no feedlot exposure period.

i. Dairy Cow Study - TCDD Transfer to Cow's Milk

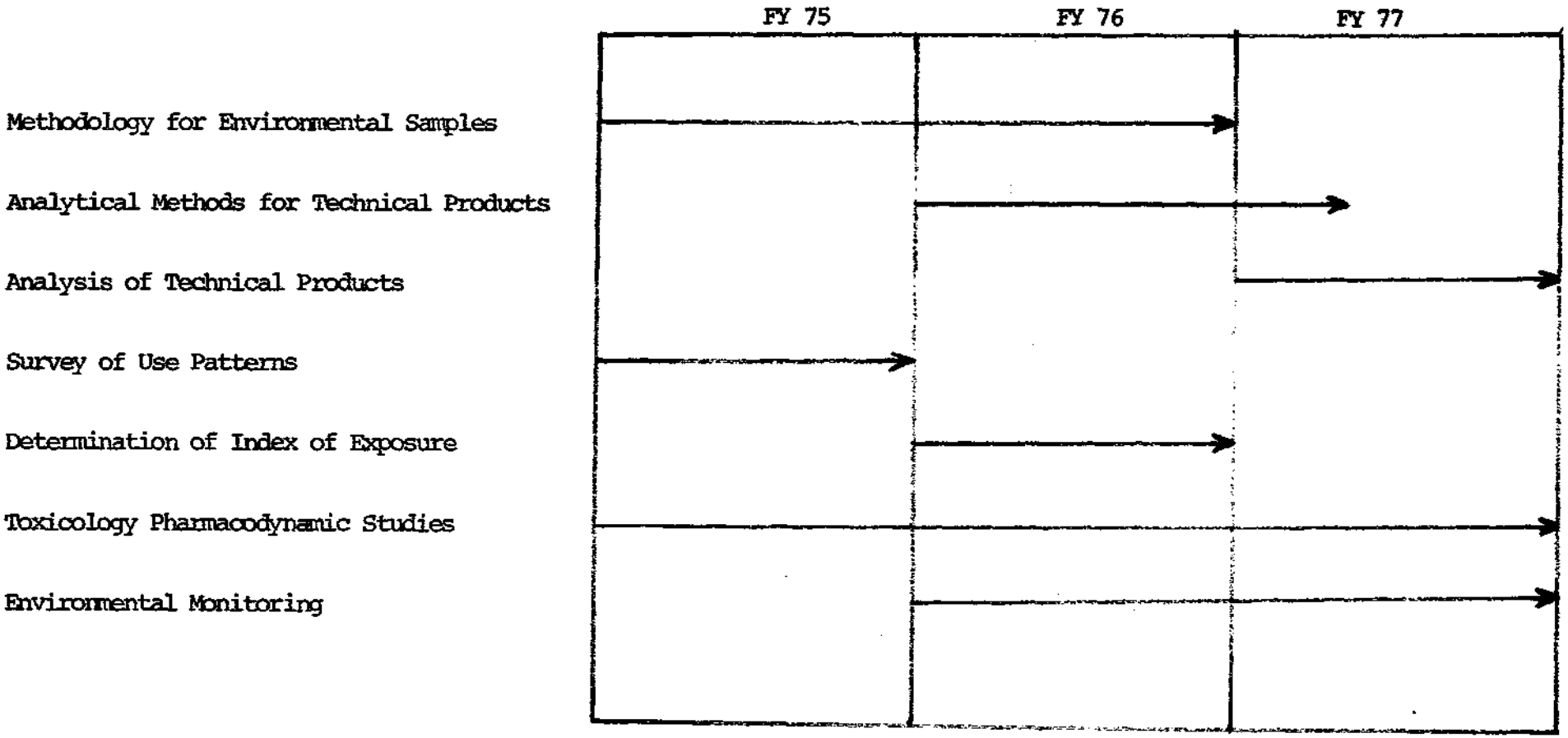
The herbicide silvex is currently registered for use on pastures and rangeland. Some currently registered products containing silvex do not have label restrictions prohibiting the grazing of dairy cows immediately after treatment. If justified by index of exposure, controlled field studies would be desirable to establish treatment and grazing conditions by which possible TCDD residues could either be eliminated or minimized.

TABLE 1

LIST OF PESTICIDES WHICH MAY CONTAIN TCDD AND THEIR PATTERN'S OF USE

PESTICIDE	USE	USE NO.
2,4,5-Trichlorophenol (antimicrobial)	Surface disinfectant	1
	Oil well recovery fluid preservative	2
	Paper and pulp mill antimicrobial	3
	Cutting oil preservative	4
	Textile finish preservative	5
	Cooling tower antimicrobial	6
Silvex (2,4,5-Trichlorophenoxy propionic acid (herbicide)	Rangeland herbicide	7
	Pasture herbicide	8
	Aquatic herbicide(lakes and ponds)	9
	Aquatic herbicide (Federal uses)	10
	Sugarcane herbicide	11
	Rice herbicide	12
	Home use (lawns)	13
	Pre-harvest fruit set control (apples)	14
Erbon (herbicide)	Soil sterilant, industrial type	15
Ronnel (insecticide)	Animal feed supplements	16
	Residual sprays	17
	Thermal fogs	18
	Banana treatment	19
	Fly bait formulations	20
2,4,5-T (2,4,5-Trichlorophenoxy acetic acid) herbidide	Rice herbicide	
	Rangeland herbicide	
	Pasture herbicide	
	Forest herbicide (pine release)	
	Rights-of-way (highway and utility)	

FIGURE I.


FY 75
FY 76
FY 77
Methodology for Environmental Samples
Analytical Methods for Technical Products
Analysis of Technical Products
Survey of Use Patterns
Determination of Index of Exposure
Toxicology Pharmacodynamic Studies
Environmental Monitoring