DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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TRANSCRIPT OF PROCEEDINGS

OF THE

INTERAGENCY MEETING ON POLYCHLORINATED BIPHENYLS (PCBs)

Thursday, August 5, 1971

Dr. Dale R. Lindsay, Associate Commissioner for Science,
Food and Drug Administration
Chairman

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TRANSCRIPT OF PROCEEDINGS

OF THE

INTERAGENCY MEETING ON POLYCHLORINATED BIPHENYLS (PCBs)

MONS 069141

ATTENDEES

COUNCIL ON ENVIRONMENTAL QUALITY

Dr. Warren R. Muir

DEPARTMENT OF AGRICULTURE

Dr. George F. Fries
Dr. Fred J. Fullerton
Dr. Philip C. Kearney
Dr. Kenneth M. McEnroe
Dr. William S. Monlux
Dr. Harry C. Mussman
Dr. Jack R. Plimmer
Dr. Robert C. Riley
Dr. Frederick R. Senti
Dr. Joseph S. Stein

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

Center for Disease Control

Mr. William F. Barthel

Food and Drug Administration

Dr. Frank N. Allan
Miss Helen C. Barry
Dr. Nichols H. Booth
Mr. J. William Cook
Mr. Paul E. Corneliussen
Dr. William D. Aguanno
Mr. Reo E. Duggan
Mr. Hyman P. Eiduson
Mrs. Mary K. Ellis
Mr. John D. Findley
Dr. Donald F. Flick
Dr. Gerald B. Guest
Mr. Leroy Houser
Mr. Kelvin H. Keath
Dr. Albert C. Kolbye
Dr. Herman F. Kraybill
Dr. Dale R. Lindsay
Mr. Donald R. Martin
Mr. Joseph J. McGuire
Dr. H. Dwight Mercer
Dr. Murrell P. Morris
Mr. Lessel L. Ramsey
Mr. Lavega R. Shelton
Dr. Kenneth E. Taylor

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Food and Drug Administration (Continued)

Mr. Robert A. Tucker
Mrs. Barbara Van Schoick
Mr. Henry L. Verhulst
Mr. John R. Wessel

National Institute of Environmental Health Science

Dr. Lawrence Fishbein
Mr. William W. Payne

Health Services and Mental Health Administration

Dr. Vincent F. Maturi

DEPARTMENT OF INTERIOR

Dr. Eugene H. Dustman
Mr. Robert G. Heath
Mr. Bernard M. Mulhern
Dr. Lucille F. Stickel

ENVIRONMENTAL PROTECTION AGENCY

Mr. Virlyn W. Burse
Dr. Morris Cranmer
Dr. Thomas W. Duke
Dr. Douglas Hamner
Dr. Renaulti D. Kimbrough
Dr. Douglas Worf

NATIONAL OCEANIC AND ATOMOSPHERIC ADMINISTRATION

Mr. Thomas C. Carver
Dr. John A. Emerson
Dr. Joseph H. Soares

OFFICE OF SCIENCE AND TECHNOLOGY

Dr. Edward J. Burger, Jr.

NON GOVERNMENT

Dr. Richard S. Gordon
Dr. Thomas J. Haley
Dr. J. R. Harris
Dr. Roger A. Hoopingarner
Dr. Norton Nelson
Dr. Lawrence M. Roslinski

P R O C E E D I N G S

DR. LINDSAY: I am Dale Lindsay, Associate Commissioner for Science, Food and Drug Administration. I apologize to everyone here for the catch as catch can nature of this meeting.

In discussing this late last week with the Commissioner, he indicated that he thought it probably would be a good idea to exchange information between the agencies that are involved. The effort was so hurried that besides Agriculture, EPA, and FDA, I am not sure whether we have other agencies here or not.

As we go through the program anyone who has anything to say -- and I would encourage you to have something to say -- please identify yourself by name and where you are from, for the benefit of our Stenotypist who is keeping a record of this meeting today so that all of us may know what happened and hopefully there won't be any argument about who said what.

There is some problem, of course, about a meeting with as many technical terms as will be used here today. I have asked the gentleman to use his best phonetic spelling and we will try to be sure that we can fill in for what he may not be familiar with.

There is no program, no agenda other than the agenda I have discussed with representatives of the agencies we have talked to. The idea is to exchange information and I thought that probably

the best way to do this would be to ask each agency with a problem to give a situation report of that agency's problems or their findings. You don't have to tell us what you think you are going to do. We will leave that entirely up to you. The idea here is to identify interests, abilities to cope and to come up with some sort of a better idea of who is doing what and what yet needs to be done and where it might be done.

I think in the interest of time, since we are running late, I would like to have Dr. Senti present the Department of Agriculture's position reports.

DR. SENTI: Thank you, Dr. Lindsay. As Dr. Lindsay has said, our meeting has been put together on rather short notice. We haven't had much preparation for anything like a formal presentation. However, we do have some people who can report on some of the things that are going on in the department. We thought probably it would be good to start out with a report on the situation that has focused attention on PCBs in the last few weeks, and that relates to the finding in our poultry inspection service.

We have asked Dr. Stein, who is coordinator of the PCB work in that inspection service, to discuss it and give you a report on the monitoring program in the meat inspection service. Dr. Stein.

DR. STEIN: As Dr. Senti has said, this was very unexpected for me to be up here this morning and talking about this. The only data that I have with me are up in the top of my head. I have been rather closely associated with this project for about two weeks now.

I think maybe if I just tell you how it came to our attention and what we have done up until now, it might be helpful to you.

We found out about this on July 19, which was two weeks ago Monday. We found out about it from representatives of Holly Farms in North Carolina, who told us that they had been having hatchability problems with their breeding flock. The hatchability was down very low in some cases.

As I gathered the information, they had done a lot of examination and testing to try to determine what their problem was. They had pinpointed fish meal and PBCs in fish meal. They then had called this to the attention of Monsanto, who I understand then contacted the Food and Drug Administration. Is that right, Mr. Duggan?

MR. DUGGAN: That's right, right after noon.

DR. STEIN: On Monday, July 19, the poultry producers' representatives were in our office talking about it. At that time I don't think anyone knew what the real extent of the problem was, although it was known that other people in the area had hatchability problems and therefore probably used some of the same feed.

I forget how this came about but somehow we got an understanding that Food and Drug was investigating the fishmeal plant and was going to furnish a list to us of the other purchasers of the fishmeal. We didn't really feel that we had enough information at that time to do anything dramatic on a national scale. We wanted to learn more about the nature of it.

We waited to get the list, which was furnished to us on July 22, Thursday of that week. It turned out there were 64 prime customers of the fishmeal plant, most of whom we could identify as being

integrated poultry producers.

At that time we sat down quickly with a small staff and decided what we were going to do. It looked like it was covering 12 states and many people. The poultry integrators, as you know, sometimes have hundreds of growers working for them individually. We came to the conclusion that it would be impossible with the laboratory facilities available and other limitations to test all the birds involved. So we decided to try to find out as quickly as we could what the situation was.

Our plan called for taking multiple samples of all types of poultry and swine from every plant that was working under federal inspection in the areas involved. In the meantime we found out that in two of the states, Arkansas and Indiana, which had been listed as prime purchasers, the plants or the purchasers in those areas did not use the fishmeal for livestock feed. One returned it because they had examined it and found it had PCBs in it and one used the fishmeal for preparing fish feed and also laboratory animal food. They didn't use it for livestock feed.

So we had 10 states involved and approximately 180 poultry plants and, as I recall, 30 or 40 swine slaughter plants. We set up our program. We told our people on Friday, July 23, to begin that day to pick up samples to send to our laboratory in Washington and to our laboratory in San Francisco. We gave them instructions on how to take the samples, pack, them, keep them in dry ice. We had our labs all primed and ready to go and the samples began arriving over the weekend.

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We began to get analysis about the 27th. During what we would call the CRASH testing program, yesterday we had had reports on 383 samples. They include turkeys, broilers, layers and hogs. We have not this morning, to my knowledge found a sample that exceeded the administrative guidelines established by the Food and Drug Administration, which is five parts PPM in the edible tissues of the bird.

We began to be a little encouraged by our findings by the middle of the receipt of the samples but we couldn't be complacent because we had not concentrated on what could be the biggest problem, and that is the breeder fowls which were the cause of the knowledge in the first place, and layer fowls, both of which are kept much longer than broilers.

In the meantime I should say we have found out that fishmeal is not a big item in hog feed. It is used to some extent but not like with poultry. So we set up last Friday a plan to concentrate on layers and breeders. We went back to every plant in the 10 states and told them to take samples from up to 10 lots daily of those that were slaughtered beginning Friday and going up through this week. They wouldn't take 10 samples if they didn't kill 10 lots, but I believe some killed that many.

Those are being sent in to our laboratory right now. At the moment we have had no results on those latest samples. We plan to do continuing testing on broilers and we also plan to do some more tests on hogs.

When this first happened we went back and checked our records of testing. We have been screening our routinely collected chlorinated hydrocarbon samples for PCB since about January. We had some problem with PBCs in another state at that time.

We found in our records I think one sample that showed low level PCBs somewhere about March or April out of a few hundred samples we had screened for chlorinated hydrocarbons. We are continuing to screen our regular monitoring samples for PCBs and as I say, we will be continuing on an expanded basis to test samples.

I don't know what we will do until we get results on breeders. That is where we are now. I didn't give you a lot of details, but I think that brings you up to date on what we have done.

Let me first ask if my co-workers want to add anything to what I have said.

DR. KIMBROUGH: Since the hatchability seems to be important, have you analyzed any eggs or are you planning to?

DR. STEIN: We have not analyzed any eggs. I think that question would better be put to Mr. Duggan.

MR. DUGGAN: Yes, we are analyzing eggs.

DR. STEIN: You probably read in the paper that a number of birds were destroyed. They were destroyed on the Holly Farm down in North Carolina and they were destroyed on the basis of pretesting of live birds that Holly Farm was doing through a private laboratory. We have found very high amounts in some live birds

present there. Some of them have been over the administrative guideline.

Fortunately, those birds having that high amount were caught alive, killed and buried on that farm without being brought to slaughter.

DR. BURGER: Would it be worthwhile to simply have quickly reviewed the incident that led to the contamination of this feed, if it is known?

DR. STEIN: I could tell it but I am not the one to do it.

Mr. Duggan could do a much better job. His people did the review. All I know is what I have read in the paper and then I talked to Mr. Duggan. I would be very delighted to have Mr. Duggan tell you about it. I can tell you what I know but he can tell you better.

Are you willing to wait until Mr. Duggan takes his turn up here?

DR. BURGER: Yes.

DR. FRIES: I was wondering if you had identified which this was.

MR. DUGGAN: I didn't plan to get into that but we have talked to the Monsanto people and they tell us, as its humanly possible, they have made that 100 percent 1242.

We have heard some rumors it might be just a portion of it but they said that particular food is as perfect as they can make it, although not 100 percent.

DR. STEIN: Are there any other questions?

Thank you very much.

DR. SENTI: Another part of the Department's activities relates to work in the state experimental stations as related to our Cooperative State Research Service, particularly the research that is going on there. Dr. Riley of our research service will give a brief review of the activities in the experimental stations as they relate to PCB.

DR. RILEY: Thank you, Dr. Senti.

I would like to discuss in broad outline some of the work in the State Agricultural Experimental Stations related to PCB.

We have pesticide residue laboratories in each of the 50 states. These laboratories are coordinated by regional projects in each of the four regions. There is federal participation on these regional residue projects.

CSRS has submitted names of key persons who are involved in this PCB situation. Time has not permitted bringing many of these people in for this meeting. Frequently progress reports shown on USDA-CRIS-project printouts do not mention the work in a specific area such as PCB.

For example, regional project W-45 has emphasized for several years coordinated research on chlorinated aromatic pesticides and PCB has received concerted attention. Dr. F. A. Gunther, at Riverside, California, has served as a clearinghouse for PCB samples to the participants of the Regional project. California-Davis is studying the photo-decomposition of PCBs. Colorado is studying the occurrence

of PCB in sewer sludge and the breakdown of PCB in sludge when it is applied to the soil. Nevada is looking at the influence of PCB on enzyme systems. Hawaii is working with the influence of PCBs in semi-tropic climate.

California-Riverside has developed a carbon-skeleton chromatography method of separating and analysing PCB in the presence of DDT (J. Agr. Food Chem. 19, 396-398 (1971)). Also they are working with the fate of PBCs in the soil. They have a soil bank of 150 soil types, and are working with PBCs in six representative soil types. Incidently, they are finding that PCBs are quite persistent in the soil.

Scientists at California-Riverside are also working with PCBs on vitamin D metabolism, calcium transport and calcium deposition in eggshells. In addition, the influence and effect of PCBs on parasite and predators, storage in fat, and biological magnification are being examined.

Similar research on PCBs is being conducted under a regional project in the northeastern region. Cornell University is concentrating on PCBs in printers' ink, in newspapers and the relationship of recycling newspapers into food containers and movement of PCBs into the food chain. There is considerable feeling that the burning of newspapers may be a mechanism that PCBs are widely distributed in the environment. The Geneva, New York station is working on PCBs in fish.

Wisconsin has rather comprehensive work in the influence of PCBs on wildlife and some very specific projects are aimed at pinpointing

these influences.

I am happy to see Roger Hoopingarner and Dr. J. H. Harris here from Michigan and North Carolina. I am sure they will tell us about the work on PCBs in their respective states.

There are a few examples of the research being conducted at State Agricultural Experiment Stations and do not reflect a comprehensive survey of the work. Moreover, much greater productivity can be accomplished within the framework of the pesticide regional projects and related wildlife and animal research if additional emphasis is placed upon this problem.

Thank you very much.

DR. SENTI: I think that just about covers in broad outline the kind of thing that is going on in the Department. We have an ARS in our pesticide residues analysis. We have been concerned with PCB as it interferes with the analysis for other chlorinated hydorcarbons but I don't know that we need to speak to that at this moment.

If we get into problems of analytical methodologies this may arise. At this point I think I will turn it back to you, Dr. Lindsay, and perhaps you can enlarge some of the questions that have arisen in the discussion.

DR. LINDSAY: Thank you, Dr. Senti, and other members of the Department of Agriculture. I think it's probably appropriate to say at this time, since I forgot to say it earlier, that you will have the privilege of all good Washingtonians of extending and modifying your remarks. We will have a record of this early next week, I believe.

We will send the record around to the participants and you can take that opportunity then to clean up anything that you may have misspoken or feel that you would like to change. When that is done, we will then distribute it to all the participants here for your further reference.

I am not trying to take notes like I normally would because I am planning on getting a much better job from our Reporter.

The logical agency to follow, I think, would be FDA. However, Dr. Duke of EPA is with us for a brief period because he has another meeting that is in progress right now, so I would like to change the arrangement around and ask others who may be speaking for EPA to see if they would like to move in at this particular juncture.

EPA has not quite reached the same acme that Agriculture and FDA have at the moment of never being able to do anything right so we will sandwich them in between the villains.

We will let Dr. Duke make his presentation now and then he can catch the shuttle.

Dr. Duke?

DR. DUKE: I am Thomas Duke from the Environmental Protection Agency, Office of Research and Monitoring, Gulf Breeze Laboratory. I would like to mention at the beginning that there is other work going on within EPA, some of which you will hear about. Also there is some other aquatic work going on at EPA's Duluth Laboratory.

I am going to speak only about data we obtained on PCBs in particular Aroclor 1254, at Gulf Breeze.

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We have had fish kills in the Pensacola area and when this occurs we usually check dead fish for pesticides residues and related compounds. In the summer of 1969, we detected PCBs in some fish that were brought in.

To make a long story short we traced the PCB, Aroclor 1254, to the effluent ditch of the Monsanto Chemical Company on the Escambia River. The contamination reportedly reached the river through a leak in the heat exchange system of the plant.

The Aroclor 1254 flowed from their effluent ditch into the river and subsequently into the sediment and animals of Escambia Bay and the water of the Escambia River. I have some slides and it might be helpful if I went over these quickly with you.

This is a map showing the subject area near Pensacola, Florida.

(Slide.)

You will notice the location of the Escambia River and Escambia Bay. The Aroclor 1254 entered the Escambia River approximately six miles from the bridge at Station No. 2. We detected it in fish, as I said, that were brought in as part of a fish kill. We do not believe that the Aroclor 1254 in the effluent caused the fish kill.

The cause of the kill was later shown to be a lack of oxygen, but at this time we did pick up the Aroclor 1254 residues. Subsequent samples showed Aroclor in other components of the system.

(Slide.)

This table shows you some of the levels of the Aroclor 1254 that occurred. These data are for croaker, menhaden and pinfish.

In another sample, flounder liver contained approximately 76 ppm, shrimp, 1.5 parts per million. In sediment from the bay we found 1.7 parts per million. Sediment from the river outside the weir at the Monsanto plant contained approximately 400 ppm.

I will now back up to the original slide.

(Slide.)

In 1969 we sampled the sediment from the river to Station 4 and we have that data. Levels of Aroclor 1254 reached approximately eight parts per million in the lower area between Stations 4 and 5. In December 1970, we conducted another survey in which we started at the Escambia River and went across the bay to the inlet to the Gulf of Mexico. At these stations we took duplicate top and bottom water samples and grab samples of sediment and also some core samples.

To give you just a general idea of the levels involved, at the Monsanto weir -- this is in December of 1970 -- we found 55 parts per million in bottom sediment. This is compared to the 400 parts per million we found in 1969.

I hesitate to compare the content of PCB in these sediments because the amount of PCB in the sediment depends upon the size and chemistry of the sediment particle. The finer organic settlements have more PCB than the sand-sized grain. We have a shifting situation up there and I am not certain that our two grab samples contained sediment of exactly the same composition.

During the December 1970 survey, we found 18 parts per million in sediment between Stations 2 and 3 (on the map) and 7.2 ppm at Station 5.

We found trace amounts, somewhere around .05 parts per million, in sediment near Station 6 and then surprisingly in East Bay, off the map here. About four miles up East Bay we found traces of PCB in the sediment and also under the Bay Bridge in Pensacola Bay.

This was in December of last year. We did find one part per million in the water outside the weir. This was attributed to leaching from the sediments in the weir. Since that time, Monsanto has shut down that effluent ditch and diverted their effluent through a new ditch.

We sampled just lately and we did not find levels of PCB in the water or at least if they are there they are below .01 parts per billion.

In December, 1970, we also took some core samples. Outside the weir, the core sample showed 78 parts per million of PCB on the surface; 30 parts per million, two to four inches below the surface; six parts per million, four to six inches below the surface; and .4 parts per million, eight inches below the surface. In Pensacola Bay at Station 5, we took more cores. As an example, one core contained Aroclor 1254 at these levels: Zero to two inches below the surface, 10 parts per million; eight to ten inches, 18 parts per million; ten to twelve inches, 1.2 parts per million. We still find PCBs at the 1 to 2 parts per million whole body range in some animals from the bay.

I think the general trend is that we are seeing some loss of PCB compared to our 1969 samples. We are also doing some laboratory work on the toxicity of Aroclor 1254.

I should mention first that we have several investigators working on this at the laboratory and I am presenting the data of several people.

(Slide.)

This slide shows some of our earlier work, just to give you an idea of the toxicity. Here we are working at 5 parts per billion of Aroclor 1254 in the water. This material is insoluble so we are using acetone as a solvent and maintain acetone controls for comparison.

You can see at 5 parts per billion we get 72 percent mortality in shrimp over a 20-day period, and juvenile fish are also susceptible. In oysters we have seen some loss of rate of shell deposition in oysters exposed to 5 parts per billion of Aroclor 1254 in the water.

(Slide.)

We also are looking at the effect of Aroclor 1254 on the estuarine fish, Spot. I thought it might be of interest to you that in tissue distribution studies, the liver contained the highest amount. This is spot exposed in the laboratory by the way.

Also, in shrimp, the greatest amount is in the hepatopancreas as opposed to the muscle. I just show this slide to illustrate again that these are the results of several analyses. If you will notice the graph, the hepatopancreas contains by far the greatest amount. We are continuing to work with this material in the laboratory.

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At this time we are involved in observing the histopathology of shrimp and fish that have been exposed to Aroclor 1254 in the laboratory.

I think that is about all the data I have to present. I will be glad to answer any questions.

DR. LINDSAY: Are there any questions of Dr. Drake?

DR. SENTI: You commented that the fish kill was not actually due to PCB?

DR. DUKE: Yes. I think we all agree that in the Escambia Bay we have an oxygen deficiency problem at certain times of the year. It was at these times of the year that the fish kill occurred.

We do not feel that the Aroclor 1254 alone caused the fish kills. We have not done the interaction experiments necessary to determine the effect of these levels of PCB in the presence of low oxygen stress.

DR. LINDSAY: Are there any further questions?

Dr. Worf has come in since we started with EPA and I would like to find out from him if he would like to have any introductory remarks to other speakers from the agency.

Dr. Douglas Worf.

DR. WORF: Dr. Lindsay has probably told you by now that this meeting was arranged in quite a hurry. We do have a strong contingent here, I think a very good representation of those individuals who are doing or have done the work and will undoubtedly do the work in this area that needs to be done.

This group from EPA are Dr. Hammer, Doug Hammer from our community health environmental group down at the Triangle, the Health Effects group. Just before the meeting he gave me series of reprints of papers that his group had done and are responsible for. I think he may have a few words to say in summarizing the work that this group has done and he will make some recommendations for the future.

Then we have Dr. Cranmer. I did not get a chance to talk to him. I just left a message for him to be here and I see that he is here. I cannot say exactly what he will have to contribute here, but I will leave that pretty much up to him.

Then we have several people who came from our Chamblee Toxicology Laboratory in Georgia. Are that lady and gentleman here?

DR. KIMBROUGH: Yea, we are here.

DR. WORF: I hope you will have something to say on this subject.

I also called Dr. Norton Nelson. I am glad to see that Dr. Nelson is here.

Is there anyone I am leaving out from the EPA establishment?

DR. LINDSAY: Why don't you go ahead and introduce them as they come up and if somebody else shows up you can take care of it then?

DR. WORF: Doug Hammer, will you come up here, and then followed by Dr. Cranmer and then the lady and gentleman from Chamblee. I will leave it up to you for the rest. I think Dr. Nelson may be able to give us contributions at any time.

DR. HAMMER: We have been concerned with PCB and our work is primarily epidemiological. We have studied PCB and other pesticide residue burdens in both urban and rural non-occupationally exposed blacks and whites living in one South Carolina County.

I do have two extra sets of these papers in the back and can have more xeroxed. I just had no idea how many people would attend today.

We studied a total of 723 people in four residence-race categories, i.e. urban blacks, urban whites, rural blacks and rural whites. As expected, PP'DDT and DDE residues were almost universal.

DDD residues were found in 84 percent of the people and Dieldrin was found in 63 percent of the people. PCB residues were found in only 40 percent of the people, with values ranging up to 29 parts per billion.

Significant age effects, not always linear were observed for all residues except Dieldrin and PCB. Racial differences were marked for every residue -- ethnic-residence interactions were also the same except for Dieldrin.

There were residence effects for all residues except DDE and Dieldrin, but sex differences only for DDE and DDT. PCB residues were more frequent and higher in whites and in urban residents.

Residues were rare in rural blacks, only 4.1 percent of the rural blacks having any PCB residues. Let me give you the mean values for them.

For PCBs there were 100 to 150 people in each group. Rural Negroes had an arithmetic mean of 0.3 parts per billion.

This again is only 4.3 percent of that population. Urban Negroes had an arithmetic mean of 1.9 parts per billion. Urban whites had an arithmetic mean of 2.3 parts per billion, and rural whites had an arithmetic mean of 3.1 parts per billion.

So if you are an epidemiologist, it is not too easy to figure that pattern out. Most of us feel that urban exposure to PCBs would be via polluted air and contaminated water. We have measured PCB in suspended particulates and they are present.

Also, we have a contract in progress for the study of PCBs in human beings and also an industrial use profile of three SAMA's in South Carolina, looking at certain aquatic and land animals and also fat and serum from persons having surgery.

Summarizing, we have in our own small way been concerned about this problem and have been measuring PCB burdens in the general population. Clearly, we did find PCB present. I think we all know possible sources of these PCBs. All of these people were asymptomatic and there is no reason to think they were ill. Nevertheless they are picking up PCB as they are many other pesticides.

Are there any questions?

MR. BARTHEL: What body fluids or tissues were used?

DR. HAMMER: These are in serum. We are also collecting about eight or ten tissues from autopsied people and we will be measuring PCB in the liver as well as the fat and serum.

MR. MUSSMAN: Have you found any correlation between the fat and serum levels?

DR. HAMMER: I do not have the values on the fat levels. The PCB levels in the serum did not correlate very well with the other DDT derivatives in serum.

MR. MUSSMAN: But in no instance were fat and serum samples selected from the same patient?

DR. HAMMER: Right. I've presented completed work. The other work is in progress now and I do not have the results and I could not get them in two days.

DR. FISHBEIN: Do you have a spectrum of the PCBs that are actually involved?

DR. HAMMER: Dr. Lamar Prietor of the South Carolina State Board of Health is doing our PCB work for us and I am truly not an analytical chemist. I know there are probably about 19 compounds that you can measure and he has worked out a fine technique using five "finger prints" and gets about 90 percent of the PCBs present.

I would be happy to put you in touch with him because he really understands the method, and I am not an expert in it. They are not easy to measure.

MR. BURSE: What techniques did you employ in order to separate the PCB from the DDT-like materials?

DR. HAMMER: He was using a methanolic calcium hydroxide digestion. This makes it water soluble, removing essentially everything but the PCB.

DR. CRANMER: I might speak to you a bit about some of the activities at the Perrine Primate Laboratory in terms of analytical methodology and toxicology of PCBs.

Dr. Frank Biros has utilized the combination of the mass spectrometer and the gas chromatograph to analyze mixtures of these compounds. Basically this concept is a gas chromatographic separation followed by the selection of a particular component of the PCB mixture for analysis by the mass spectrometer. One can take quantitate complicated PCB mixtures by selectively examining individual components, separated by gas chromatography, with the mass spectrometer. There are components of these rather complicated mixtures which are peculiar to or particular formation of PCBs and these are the components of special interest. If you have a mixture of several PCB formulations, which you will often find resulting from general environmental exposure, you may approach representation of body burdens in the general population of the country. Quantitation of this type of sample would be extremely difficult because of the many components represented in various concentrations.

If one applies the technique of a gas chromatographic separation and the application of the mass spectromenter to the examination of chlorine ratios and fracture patterns an approximation of the relative contribution of the various PCB mixtures can be accomplished.

Dr. Enos, Chief of the Chemistry Branch has been responsible for a program for analyzing human tissues for several years with some considerable success. In cooperation with the Division of Pesticides and Community Studies, he has examined several tissue samples from humans which were extremely high.

One technique that is showing promise in our laboratory is using capillary columns in the gas chromatograph prior to the mass spectrometer. This gives extremely good resolution. One does not have quite the problems with the PCB analysis by the mass spectrometer that were experienced with some of the chlorinated hydrocarbon pesticides which decompose on the gas chromatographic columns and on the separators.

We have had a considerable success when dealing with analysis in the presence of chlorinated pesticides. If one has a sample with DDE and DDT or several other compounds in the body fat perhaps even from some sort of drug therapy the problem is immense in terms of trying to ascertain the exact exposure to any particular formulation, if no selective criteria is applied.

However, with PCB analysis just as with other types of analysis there is no substitute for good clean up procedures.

Of course, I am not saying it is going to be easy. You can tie up a gas chromatograph and mass spectrometer combination for two or three days analyzing one complex sample if you want to do it accurately. However, at the Perrine Primate Laboratory we are now including analysis for PCBs in our pesticides residue chemists' training course as part of the standard procedures because these compounds are showing up in so many human tissues in the monitoring program.

I would like to say just a few words about the experimental animal work that we are doing on the interactions of PCBs on the toxicities of certain pesticides.

One case that you may be interested in is a vitamin C-deficient guinea pig. You get a fourfold decrease in the LD_{50} of 1260 in ascorbutic guinea pigs.

A diet of 50 parts per million of 1260 in the diet of a rat, decreases the fat storage of lindane by 75% probably through microsomal enzyme accumulation but slightly increases the storage of p,p'-DDT.

This is interesting since here we have two pesticides, both chloinated hydorcarbons, being effected in opposite directions in terms of their storage. The toxicity of the organophosphate insecticide parathion is altered in the vitamin-deficient animal when you pretreat with 50 ppm 1260. You change the ratio of the metabolites of parathion that are formed. Parathion, is metabolized by two major routes. One is the direct detoxification and formation of diethylthiophosphate and p-nitro phenol. The second pathway first oxidizes parathion to paraoxon which is then further metabolize to diethyl phosphate and p-nitrophenol. Then you break that up to diethyl phosphate and other materials.

With pre-treatment with PCBs you get an increase in the parathion to paraoxon metabolism rate. One might at first expect if you were getting more paraoxon that you should produce a more toxic situation.

The rate of cholinesterase inhibition is increased in animals pretreated with 1260, due to the more rapid conversion to paraoxon but the total depression of cholinesterase is less since the biological half life is shorted due to the simultaneous increase of the direct detoxification pathway.

In summary, I would like to emphasize that PCBs have many interesting effects, on the metabolism and toxicities of pesticides and other compounds and one should avoid generalizations about the toxic effects which have yet to be described.

Thank you.

DR. HAMMER: I just wanted to mention some work that is being done not supported by us down in Charleston, South Carolina, where PCB at levels of 0.001 to 0.1 parts per million has been concentrated up to 200,000 times in coli.

DR. CRANMER: That is interesting. I think the amounts of DDT required for no effect on your liver microsomal enzymes are approximately the same in the immature rat. I think you can draw some correlation.

DR. WORF: I think next we will have Dr. Kimbrough and next her associate, Mr. Burse.

Dr. Kimbrough is from our Toxicology Laboratory in Chamblee, Georgia.

DR. KIMBROUGH: Thank you very much.

We have studied the toxicity of 2 PCBs, Aroclor 1254 and Aroclor 1260 in the Sherman strain rat.

Let me speak first of all about Aroclor 1254. The acute oral LD_{50} in adult rats is greater than 4 grams per kilogram. We also undertook a subacute feeding study in both male and female rats which included a reproduction study. I will just give you some of the results that we have now and if there are any questions I do have some tables that I cannot present but I can look up some of the facts.

We found that feeding 500 parts per million to both male and female rats over a period of about 240 days lowered the hemoglobin and the hematocrit. These animals also showed less weight gain.

As far as the reproduction study was concerned, a definite effect on reproduction was produced at 100 parts per million. We did the first breeding after 76 days on the treated diets and we noticed this effect then. There were less offspring. The offspring at weaning were smaller and the survival was decreased.

We did study a lower dose, 20 ppm, and noticed no definite effect on reproduction except that the number of animals born was slightly lower. When we did autopsies on these animals we found that everything from 100 ppm on up showed an increase in the liver weights. Most of the other organs were more or less normal. There were some exceptions which I will mention later.

We also found that some animals had porphyria. We found that in the male rats fed Aroclor 1254 it was produced in some animals at a dietary level of 100 ppm (approximately 5 mg/kg per day). There was none at 20 ppm. In the females we found fluroescence of the urine as well as the plasma at 20 ppm. We are going to look into this more.

Sometimes neither plasma nor urine fluoresce but a pigment will be present in the liver -- we have also had some rats where we found fluroescence of the teeth and other organs. It doesn't occur in all the animals. It occurs more in females than in males.

We also performed autopsies on the offspring. We found an increase in liver weight in the F_{2a} generation of the weanlings at a dietary level of 20 ppm.

We also gave some rats several dosage levels of Aroclor 1254 during pregnancy. The dose that caused an affect was 50 mg/kg/day given during the 7th to the 15th day of gestation.

Giving the material dermally at the dosage level of 50 mg/kg/day for 30 days an increase in the weight of the livers of the exposed animals was observed.

I would like to give the toxicity data on the Aroclor 1260 now. In some of the animals that I have mentioned Mr. Burse has determined PCBs in various tissues and he will present his findings following this report.

The Aroclor 1260 seems to be less toxic. We found that the oral LD_{50} was more than 10 grams. At a level of 500 ppm the hemoglobin was affected. The body weight was affected in the animals that were fed on a diet of 500 or 1000 ppm of Aroclor 1260.

The livers were increased in size at autopsy. The livers were larger and heavier from the dietary level of 100 ppm on up in male rats. In the females in this feeding study the liver weights were not significantly increased at any of the dietary levels that were tested. The reproduction, with 1254, was definitely affected at 100 ppm. With 1260 it was not affected at 100 ppm but was affected at 500 ppm if the Aroclor 1260 was fed over a period for 76 days.

We again found fluorescence in the urine in some animals which indicates porphyria. We found it in one of 10 males at 100 ppm

in the urine and in five of ten females at 100 ppm. We found none at 20 ppm in either sex. But with the 1254 we did find it at 20 ppm in some female rats. We also dosed some animals with various dosage levels of Aroclor 1260 daily during the 7th to the 15th day of pregnancy. The dosage level that gave an effect was 100 mg/kg/day during that period. The number of the offspring born and the survival of the offspring was reduced.

The findings that were made when the liver was studied can be divided into different parts. One is that the liver cells are enlarged in the animals that have received Aroclor over a period of time. This occurs with 1260 as well as with 1254. In addition accumulation of fat is found in the livers. Some of the changes that have been described with chlorinated hydrocarbons such as the inclusions within the liver cells and the so-called margination can also be observed.

At the higher dosage levels (1/10 males and 6/10 females at 100 ppm Aroclor 1254 in the diet for about 8 months, 1/10 female rats at 100 ppm Aroclor 1260 in the diet for 8 months, none of the males, none at 500 ppm, but several at 1000 ppm) a very pronounced change is found which has been termed adenofibrosis. There are different opinions on what adenofibrosis represents. It consists of fibrosis in the livers concomittant with a proliferation of cells which by some are considered to represent proliferated bile ducts. Others feel that they represent undifferentiated hepatocytes which proliferate when destruction of differentiated hepatocytes occurs.

In many of the livers I also observed a brown pigment which I assume is porphyrin. We did find fluorescence in these livers at autopsy.

Occasionally other organs showed changes. In some animals hyperplasia of the thyroid was observed. The incidence of bladder cancer in our rats must be very low, but I do not know what the actual incidence is. The animals are random bred. I have worked at the Toxicology Laboratory since 1962 and I have until now never seen a rat with bladder cancer, but this doesn't mean that they could not occur spontaneously. I did observe two bladder cancers in animals that were fed 100 ppm of the Aroclor 1260. The significance of this finding is doubtful at present. However, it should be investigated whether PCBs have any carcinogenic potentials.

Now, could I have the slides, please?

This first one shows the liver of an animal that has received Aroclor 1254 over a period of 8 months. You can see the enlargement of the liver cells. You also see quite a number of mitotic figures. Here is an inclusion in the cytoplasm which resembles inclusions observed in the cytoplasm when rats have been given high doses of chlorinated hydrocarbons. The PCBs seem to cause similar changes in the liver.

In the next slide you see a normal liver of a control animal. The next slide shows another stain. The previous two slides were hematoxylin eosin stains and this is a PAS stain. This shows the so-called "duct formations" which is part of the adenofibrosis. They are lined by hepatocytes which are reduced in size.

The next slide, shows a very good illustration of the adenofibrosis. Note epithelial cells that line duct formations which sometimes contain a cellular debris. Increased fibrosis which is shown in this area is also seen.

The next slide is an illustration of beginning fibrosis. Some hepatocytes have dark nuclei and some of the "duct formations" which contain a mucinous material are also seen.

The next slide illustrates again the adenofibrosis which sometimes can form nodules in the liver. It has happened that they have been called tumors but they are not. It is a reactive proliferation that can occur in the liver when liver cells are destroyed.

The next slide shows a fat stain which demonstrates the amount of fat found in these livers. I have done a little work with the electronmicroscope. We are planning to do some more work with it.

Are there any questions?

MR. BURSE: Mr. Chairman and ladies and gentlemen, I am Virlyn Burse from the Atlanta Toxicology Laboratory, Atlanta, Georgia.

The chemistry section of the Atlanta Toxicology Laboratory has been analysing blood, tissues and some excreta for residue levels of PCB derived materials with emphasis on Aroclor 1254 and 1260 using gas chromatography in addition to other systems.

We have evaluated three approaches to the separation of PCB from DDT-like materials, namely the TLC approach, using silica gel as an adsorbent, the Reynolds procedure, using florosil as an adsorbent and the Armour Burke procedure. See Appendix I for the complete manuscript.

In the mass spectrometer work that dealt with tissues our work paralleled the mass spectrometer work that Dr. Bagley reported concerning Aroclor materials in bald eagles.

There is something in the urine of Experiment C (Appendix I) that I think is worth mentioning. We combined selected samples from the urine study and examined them by mass spectrometer and found the usual chlorinated cluster. We found clusters at 254 mass units, at 288, at 324 and at 358. There was an isotopic cluster which was indicative of four chlorines that we found at 304.

Initially we were trying to figure out what could possibly give a molecular weight of 304 and have an isotopic cluster which was indicative of four chlorines. We know that it cannot be polychlorinated biphenyl. About two weeks after this work was done I was looking through Food, Cosmetics, and Toxicology and found a paper by Dr. Vos from the Netherlands. This work was a follow-up on some previous work that he had done with chicks published in Toxicology and Applied Pharmacology.

He was looking at the Aroclor 1260, the German product, the French product and the American product. He found discrepancies in the effects of the dosage levels of these three compounds. The two foreign products fall more in line together and the Monsanto product was quite out of line, with the total effect of the Monsanto product being just signs of porphyria.

He assumed there was something in the two foreign products that resulted in the anomalies he was seeing. After he fractionated these two foreign products on florisil he was able to find the tetrachlorodibenzofuran and the pentachlorodibenzofuran as well as some chlorinated naphthalenes.

The 304 does fall in line with the tetrachlorodibenzofuran. However, we have not been able to confirm one way or the other that this is 304. I have taken the Monsanto product, Aroclor 1254, and fractionated it from florisil, looked at the elution patterns by conductometric detection, and eluted until all the PCB pattern was gone but have yet to look at it on the mass spectrometer.

This is work that is pending. We have also done some work relating to reproduction studies with Aroclor 1254 and 1260. (Appendix II) We plan to continue efforts to isolate constituents of Aroclor 1254 in the standard as well as isolation of constituents of Aroclor in body fluids to determine what is responsible for the effects resulting from acute and chronic exposure to Aroclor.

Additional work will be done to determine steady state values for Aroclor at a high level as well as low. Two additional procedures using TLC will also be evaluated for separating PCB from DDT-like material. One involves chemical degradation of DDT-like materials and the other involves triple development on TLC using Kieselguhr which is a method that Dr. Vos from the Netherlands has published in the Bulletin of Environmental Contamination and Toxicology.

DR. WORF: Thank you very much.

I think Dr. Lindsey has suggested we have a coffee break.

Before we do this I would like to thank the EPA people who made the presentation here on very short notice. I think this is good hard data.

There are some gaps in the EPA program that we could not include. We do have a strong program in monitoring this type of a chemical in the environment, both in the water and in the air

and in the soil and in tissue. The tissue part was pretty well described here today.

Dr. Prindle of our Water Hygiene Lab unfortunately is not here and has had considerable experience in monitoring and developing techniques and sampling techniques and instrumentation for determining PCBs in water.

With this I think we will take a coffee break.

DR. LINDSAY: I would like to suggest that, as a possible way of getting some of this technical and detailed information more completely disseminated, perhaps we can summarize from the podium and then, if its all right with the people who are providing it, we can amplify this for the record. We will try to get distribution to everybody that is here without jeopardizing publication prospects for it later.

This is strictly an interagency type of exchange and I don't think this will constitute jeopardy for the later publication or acceptance by some critical educators.

We will take a short coffee break and be back here by quarter of eleven.

(Short recess.)

DR. LINDSAY: As I indicated earlier, there is no agenda. We are trying to play it by ear so I will reiterate what the "no agenda" is. We still have several people to hear from in the Food and Drug Administration and there is a gentleman from the Department of Agriculture that we would like to hear from. Then we want to hear from some of the people from outside the Government who are working in this field

of PCB research who can review some of their work, particularly as they relate to the problems of the regulatory agencies. They may have other areas that would be interesting, but we are particularly concerned about how their work fits into some of the problems that we have.

Over on the chairs against the wall you will see two or three piles of Xeroxed papers. The larger piles consist of what we call "status reports." Dr. Herman Kraybill is primarily responsible for getting these together in the Food and Drug Administration. We don't apologize for them but they are out of date. The June 1970 and the supplement of December 1970 are there but they are a little bit old now, the way PCBs have been moving. We don't have enough to go around. We would appreciate it very much if each group of people would not take copies for everybody but get only what you need.

I would like Mr. Duggan to give a situation report with regard to the Food and Drug Administration.

Just before we go into the activities of the Bureau of Veterinary Medicine I would like to ask Dr. Senti to introduce a man he discovered was here who could lead into some of the animal feed problems.

MR. DUGGAN: It is nice to see so many familiar faces. We had a meeting on PCBs about a year ago and a lot of you were at those meetings where we were going to talk about emerging problems. Since that meeting we have had problems emerge more than once.

Now we are right in the middle of one of the emergencies. I think I will start off by giving the FDA regulatory policy on PCBs in foods. It is a very simple one: we do not approve of any foods containing PCBs, nor do we condone any practices which result in any levels of PCB residues in foods.

While that may be our policy, until there are some legally sanctioned uses, we have been forced to compromise that policy to the extent that we do not object to the marketing of certain specific lots of food that might contain some low levels of PCBs. I do want to stress the difference between approving and not objecting to. I think this is the key.

In this particular instance of poultry we have advised the Department of Agriculture that we will not object to the marketing of poultry that contains 5 parts per million in the edible portion or 5 parts per million in the separate fat. That means that any time the fat is separated out during slaughter or processing as a finite substance to be used in other food products the 5 parts per million guideline will apply.

In terms of animal feeds we have advised them that the use of feed ingredients contained in detecteble levels of polychlorinated biphenyls for food produced in animals is neither approved nor condoned by the FDA. Such adulterated feeds can be used for animals not used for food purposes provided it is not toxic to those animals, or for non-food purposes such as fertilizers or industrial chemicals.

We have had this problem face us a number of times over the past three of four years. We have had a couple of instances where we were pressed for guidelines by the states which had discovered PCB

residues in milk. We gave them a guideline of .2 parts per million to deal with the particular situation.

The preliminary thrust of our interest in those instances was to discover how the PCBs were getting into the milk and how it could be prevented. In one instance I think it was pretty well established that the silos had been painted with a paint containing PCB.

The states did take action and they kept that milk off the market. In another case I believe it was pretty well established that one of the power companies had disposed of excess transformer oil in spraying their rights of way and dairy herds had been grazed on those rights of way.

More recently we had had this problem of poultry in the State of New York. Through the activities of the state and the Department of Agriculture the major poultry industry in the State of New York I think properly contained the problem of marketing PCB contaminated poultry and eggs.

I do not believe we ever finally nailed down the source of that contamination, though there was some indication that it might have been due to the practice of grinding up bakery products without taking them out of the packages and throwing the whole package into the animal feed system.

This brings us down to the present problem.

Friday afternoon about 5:15 on the 16th of July the Monsanto Chemical Company called and said they just wanted to tell me that there was an awful lot of fish meal in the State of North Carolina that was contaminated with PCBs and that the company

involved was the East Coast Terminal.

With fish meal being used as an animal feed and with the accumulative effects into the fat of animals, we thought perhaps we had better get the boys out again on Saturday and Sunday to investigate the problem because at that time it was only a rumor. You know, you get a lot of misinformation over the telephone.

By Monday morning the Atlanta Regional Office had visited the terminal. They found that the fish meal that was ready for shipment was contaminated. It contained anywhere from 12 to 20-some-odd parts per million PCBs. You might wonder what PCBs were doing in a fish meal plant to begin with.

Fish meal is often contaminated with salmonella. We object to the use of salmonella-contaminated fish meal in animal feeds. So the companies have adopted a policy of pasturizing the fish meal. In this case it is done by passing the fish meal through a system with the heat exchange fluid in the hollow screw. It seems that fish meal has a facility for eating holes in these screws.

After some investigation we found that this had probably started occurring about the end of April and they had been fixing the screw off and on during the process. Later on it developed they had refilled or used about 5,000 pounds of aroclors in the month of June. We found that they had shipped about 16,000 tons from the end of April until the 16th of July. We did request embargos by the state organization and began to list shipments.

The manager of the plant did not seem to have a whole lot of authority. This is why Monsanto finally called us up. They

had known about this for several days. They had tried to sell the company some different heat exchange fluids. The firm did not pay a whole lot of attention to them not realizing the significance of the problem.

The actual operation and direction and policy for this particular plant is vested in the South Pacific Protein Company headquartered in Darien, Connecticut.

As Dr. Stein has mentioned to you, the Department of Agriculture learned of this on Monday, about the same time that we had gotten our results in, and they were asking for guidelines on the poultry that might be contaminated. We had had a guideline for the New York incident, but we had told them it was not applicable to any other situation. This then meant that we had to get our scientists, our Bureau of Foods and Bureau of Veterinary Medicine, involved to take another look and see what guidelines we could use in this particular case.

The manager of the South Pacific Protein Company came down to talk with us on Tuesday. He immediately saw the gravity of the problem, particularly in light of the fact that this is used at about two percent in poultry feeds, which means that there could be well over half a million tons of contaminated poultry feed in the system. He stated that he would have to go back and discuss what he was going to do with the plant manager, who happened to be in Peru at the time.

Apparently this is an integrated operation too, and these firms are owned by Peruvian interests. They are not, as far as

we can determine, a part of the Peruvian Government.

At any rate, early Wednesday morning he advised us that he had issued telephone instructions to all of his consignees asking them to return all unused fish meal to the North Carolina plant and that they were to ask their customers to return all of the fish meal to the North Carolina plant. This recall is going satisfactorily.

We are now engaged in looking at the eggs that might be produced from this. We are also looking to determine what happended to the eggs that did not hatch, whether they got mixed up into the incubator reject rack or if they went for industrial purposes as they are supposed to go. This is pretty much where we stand at this particular moment.

I think one thing we must recognize here is that we are not dealing with quite the same problem as we are dealing with in the pesticide area. There have not been the widespread direct uses of PCBs on food crops or in food establishments. This is a fact. Unfortunately it has fanned out into a major problem.

We are not yet at a point in our toxicology information where we can establish a guideline for all foods. I think we are going to have to continue to deal with these other circumstances involved in each case. It is not an entirely satisfactory system, but until we have a better system I think we are stuck with this one.

I think that is about all I have. I will be glad to try to answer any questions anyone might have.

MR. BARTHEL: What levels did you find in eggs?

Mr. DUGGAN: We have not put that together yet. We expect to have a fix on that about the latter part of the week In speaking of this, as you know, PCBs will be detected in the ordinary pesticide multi-residue method. Since our meeting last year we have been very much aware of this and have been looking at the chromatograms during the routine surveillance of our food for PCB residues.

I have enough confidence in the ability of our field laboratories to believe that if there had been any substantial amounts or abnormal amounts, let us say, approaching detectable and measurable levels of PCBs in egg samples generally we would have heard about it long before now. We do not believe there should be any detectable residues in eggs.

This works out, I understand, to about a .5 parts per million guideline.

DR. BURGER: Just to make sure I understand, there is now an administrative guideline for PCBs in animal feed, in milk and in fish; is that right?

MR. DUGGAN: No.

DR. BURGER: I am sorry.

MR. DUGGAN: We have no guideline. There should be no detectable levels of PCBs in animal feeds. There is a guideline for milk. There is a guideline for fish. There is a guideline for poultry. Now, these are interim guidelines. They were set to deal with specific instances. If Joe Stein calls me up next week

and tells me he has a problem with PCBs in turkeys in California, for example, then we are going to get the circumstances surrounding that particular instance and then we will look at it in terms of the new toxicology we might have, how it could have been prevented, and then deal with it on that basis.

In other words, these guidelines that we have talked about here today are not across-the-board.

DR. BURGER: Is there a systematic scan for PCBs in fish at this point?

MR. DUGGAN: Yes, they will be picked up in the multi-residue methods that we commonly use for pesticide residues.

DR. BURGER: Although you say they are not necessarily specifically for PCBs?

MR. DUGGAN: The fingerprint for PCBs is quite identifiable and recognizable. I think there may be someone from the Bureau of Foods who can talk more specifically to that point.

DR. BURGER: A final question. Did I understand you to allude to an industrial use of eggs?

MR. DUGGAN: Yes.

DR. BURGER: What is that?

MR. DUGGAN: I think they use eggs in the manufacture of tanning, preparations for tanning leather. This is one use. I suspect there are other uses as well.

DR. BURGER: Thank you.

DR. LINDSAY: Dr. Senti, would you introduce our next speaker.

DR. SENTI: We have Dr. Fries here of our Animal Science Research Division who has had some experience with the feeding of PCBs to livestock and he will give a brief summary of that work.

DR. FRIES: I will cover this very briefly. Last summer I was called in to give advice to a farmer who had been taken off the market because of excess DDT in milk. I decided to use this as a field study. It was a short time thereafter that I picked up the strange peaks which we identified later as PCBs. We were certain of the identification because the Baltimore FDA laboratory had found PCB in the milk of this farm previously. The source of PCB was silo paint.

By coincidence the cows had been taken off the contaminated silage at about the same time we removed them from the source of the DDT contamination. We were able to follow the levels of both contaminants for a six month period. We sampled the cows individually every three weeks through the decontamination period.

When a cow is removed from PCB contamination, there is a 50 percent drop in the level of the milk within about the first three weeks. This is very similar to DDE. Then there is a slow rate of decontamination as the PCB is removed from body fat. This rate is about 1.3 percent per day. This is identical to the DDE. In fact, any grouping of cows you take -- individual cows varied very little but on any average group of cows the two compounds behaved identically. That pretty well summarizes the field work.

We are now feeding Aroclor 1254 to cows at Beltsville to establish relationships between the input and excretion in the milk.

Arochlor 1254 has been used in silo paints and is probably the biggest PCB problem facing the dairy industry. I think this problem is possibly bigger than has been suspected. I think there are quite a few of these silos around.

I might also mention that Dr. Bitman's group at Beltsville is doing work on PCB with rats and quail. They are studying effects on egg shell thickness in quail, vitamin A storage, and effects on the liver enzymes. I am not prepared to talk about their results.

I do have a mimeographed summary of this field study. If someone is interested in the details they could write me for a copy.

Are there any questions?

DR. HOOPINGARNER: I was curious if activated charcoal would help reduce this quicker?

DR. FRIES: No. In fact, that was part of the study. I have a disagreement on this subject with one of your colleagues at Michigan State. That is part of the study and it is included in the mimeograph. They had activated charcoal on the farm before I became involved. We fed activated charcoal in combination with phenobarbital as well as phenobarbital alone. Neither treatment had an effect on the rate of decontamination. I would suspect that if somebody had contaminated silage and fed activated charcoal with it, they would reduce the PCB level in milk by at least 50 percent or more.

I have looked at a number of other farms having these silos. In general it is a borderline situation. Some of the time

during the silage feeding period the milk level is under the guideline and sometimes it is just slipping up over it. The levels are reasonably comparable to the levels that have been reported in Ohio where they have been taking some dairymen off the market.

DR. STEIN: In running your field survey did you do any biopsies on those milk cows?

DR. FRIES: I didn't do biopsies on the field survey. I am doing biopsies on the study I have underway at Beltsville.

DR. LINDSAY: Thank you, Dr. Fries.

Dr. Mercer, are you going to speak for the Bureau of Veterinary Medicine? Will you tell us where you stand with it now?

DR. MERCER: As with most programs which have a very loose agenda, if you are able to sit back long enough, whatever you have to say will be said, so I don't think this will take very long.

I was not necessarily prepared to give the position of BVM on the PCB problem. I was more or less prepared to talk about inavailable toxicity information relating to domestic animals.

Two of the major concerns of BVM, of course, are, one, the safety aspects of PCB to food producing animals, as well as other animals that might be consuming PCBs and a second major factor that gets a great deal of attention is the tissue residue aspects in the food producing animal, as might be obtained through the feed sources.

Fortunately you have just heard the data from Dr. Fries that probably gives us as much information as is available on dairy cows. I think it is well established that for a long time we may have been

measuring PCBs in milk and calling them DDT or other chlorinated hydrocarbons.

I am going to briefly bring us up to date on the information that we do have available on the toxicity of PCBs in domestic animals and poultry. There are a couple of papers that give a very good review of what is known about the toxicity of PCBs.

Dr. Lindsay referred to the status report that Bureau of Foods, FDA, has prepared, and which is available, which covers the literature up until the latter part of 1970. There is no point in going into a detailed discussion of this published information.

A recent review article by Peapall and Lincer, the only one I am aware of, does review the toxicity of the PCBs and was published in 1970. Both the FDA status report and the Peapall article will serve to give a very good background with regard to what is known about PCBs.

There have been some toxicity studies conducted in poultry. I believe the classic work was done by McCune et. al. 1962. I will briefly give you a summary of their findings. McCune fed chickens four levels of PCB; 100, 200, 400, and 800 parts per million as a component part of the diet.

The chickens at the 100 parts per million level showed no effect on the liver. They only showed a slight fluid accumulation in the pericardium. The bird with the 200 parts per million level showed no liver effects. They did show a more extensive hydropericardium. As they went up the scale, the 400 and 800 parts per million in diets they began to observe more gross toxic effects on other organs as well as quite extensive hydropericardium.

There was also some work reported by De Nos and Kolman, 1970 on chickens receiving 200 and 400 parts per million for two weeks with a 60 percent chlorinated PCB. They found some effects at both of these levels; kidney effects at the 200 parts per million level were extensive with hemorrhage and enlargement of kidneys occurring at the 400 parts per million level. They also saw hydropericardium. They found some enlargement of organs such as adrenals and spleen. The pancreas was also effected.

Beyond this work in chickens, the information becomes very scanty. We will be hearing a report from Dr. Harris of North Carolina, who is currently involved in some toxicity studies with chickens, and I believe he will be reporting on what he is finding. This information will be most pertinent at this particular time.

A number of studies have been conducted on wild life and there have been a few toxicity studies done on ring doves where they have noticed some effects of PCBs. However, none of these studies really give us much in terms of a dose-effect correlation. This is the type of correlation that we need most in order to start thinking about regulatory guidelines. What kind of a tolerated dose can we afford to have.

Dose effect correlation data is just not available. When you get beyond poultry, and get into the swine or calves or sheep or any of the other domestic animals with which we are concerned regarding food production capabilities, toxicity and residue data gets extremely scarce. I do not know of any information on these other species at

this point. If there is any current research going on in these areas I would be most happy to learn about it. It is certainly not in the literature.

The other aspect that we need to have more information on is the tissue distribution and excretion pattern of PCBs. The best article that we can refer to in terms of published information on the metabolism of PCBs is an article that Grant et. al. published in which they did a metabolism study on rats. Again we run into this hairy question of extrapolation from rats to other domestic animals. They did report some interesting literature, including evidence that PCBs were being found in human milk. This information has been substantiated in dairy cattle where measurable levels did occur in milk.

But basically I think the Grant work confirms some previous data that was given to us by EPA people this morning and that is that the primary organ of concentration in the rats was the fatty tissue.

If you compare on a relative basis fat to serum, assuming the serium has a value of 1, the data, in general, that Grant published showed something like 508 parts per million in fat compared to 1 part in blood.

The next major order of concentration was the liver, 59 parts per million compared to 1 in blood.

The brain tissue, being the next in the sequencial order of concentration, then the kidney, spleen, heart, testes and no muscle tissue showed measurable residues.

I am not aware of any dose concentration correlation studies in the muscle tissue of birds or any other animal, as far as that is concerned. I think this is an area that we must become increasingly concerned with if we expect to have any impact on the regulatory aspects of PCBs. There is no further toxicity or residue data we can talk about in domestic animals.

We would be happy to entertain any questions you may have.

DR. GORDON: In your mind does the Bureau of Veterinary Medicine think that the outbreak of hydropericardium that occurred in early 60's, is that associated only with PCBs or in the Bureau's mind is that associated with other factors? That was a major problem in the poultry industry at that time.

DR. MERCER: I think in answer to your question that we will feel much more comfortable as some of the data that is currently under way and the results become more available to us. Again, I refer to the work that is going on in North Carolina. I think we will have some confirmation as to whether or not this was a sole effect of PCB. At this point all we have to go on is reported literature and you have to assume that this is what the major effect was.

DR. FLICK: I was wondering if Dr. Gordon's question was pertaining to the chick edema disease which occurred in the late 50's and early 60's. This disease was not due to polychlorinated biphenyls. The evidence thus far indicates that these are polychlorinated dibenzo-paradioxins and indeed are quite toxic. The polychlorinated biphenyls are toxic and produce a syndrome which we might call dioxinosis. However,

the levels of intake are much higher, several hundred fold to what we observed with the polychlorinated dibenzoparadioxins.

DR. KIMBROUGH: I would like to add something to this. The work by Vos and his coworkers has been cited several times. These authors pointed out that they found hydropericardium which is the chickedema disease in the chickens that they fed with PCBs They found chlorinated dibenzofurans as a contaminant in the European PCBs that they studied. They suggested that chickedema could also be produced by the chlorinated dibenzofurans.

DR. MERCER: Thank you for your contribution. That plus the diamines gives us a real problem.

Are there any other questions?

If not, thank you.

DR. LINDSAY: Thank you, Dr. Mercer.

Dr. Kolbye, can you tell us a little bit about the Bureau of Foods?

DR. KOLBYE: I will comment briefly and then act as M. C. for some of the other staff.

I woulk like Mr. Les Ramsey if we can get the projector to work to put on the screen some of the residue data to give you a quick idea of what we have found in certain food sampling.

Then I would like to ask Mr. Cook if he has anyting further to add to the chemistry and then ask Dr. Kraybill to tell us what are some of the gaps in our toxicological research.

Les, why don't you come up and just give a rumthrough on these?

MR. RAMSEY: The film looked as if it would not be visible to most of you. Perhaps I can summarize it, although trying to summarize residue data orally is not very satisfactory.

What I want to do is to indicate what our residue findings for PCBs have been in the past year, the period from July 1, 1970 to June 30, 1971.

We have looked for PCBs in all the samples that we screened for pesticide residues. In our pesticide surveillance and enforcement program every sample that is analyzed there is also screened at the same time by the multi-residue method for PCBs.

In this program in the past year we looked at 570 samples of fish. 315 of those samples were positive for PCB. The overall average is of the order of 2-to-3 parts per million. In other words, the fish that we analyzed, at least, do not run as high in PCB as the fish do in total DDT, that is, DDT plus DDE plus DDD.

We have analyzed 1,193 samples of cheese and found 81 positive. The highest level was 1 part per million, and the average about 3/10ths of a part per million.

We have screened 805 samples of milk. Of those, 60 were positive and were identified with the contamination incidents that you have already had described for you in the West Virginia area, in the State of Ohio, and I believe in the State of Kentucky and perhaps some of the other states of our Atlanta Region.

Some of those samples ran as high as 28 parts per million PCB in the fat in this West Virginia incident that Mr. Duggan described,

where the transformer fluid was apparently used as a solvent for herbicides used to control weeds on the right-of-way there.

Let me say that the samples that have been found positive in milk have been looked at and the situation investigated.

Usually it has been due to some accident such as the feeding that was discussed with regard to the silage in the silos painted with paint containing PCB or arising from the pasturing of land that had PCBs sprayed on it for one reason or another.

317 samples of shell eggs were looked at. Eighteen of those were positive for PCB. The highest level found was 5/10ths of a part per million. The average, of course, is much lower.

We have found traces of PCB in the by-product from two potato-processing plants. One was in the Denver District and the other in the Seattle District.

The waste coming from those plants showed traces of PCB. And so far as I know we have not yet been able to identify the cause of the PCB in the waste.

We have looked at a number of oysters. And, incidentally, oysters, by and large, do not have high levels of PCB, at least the ones we have looked at.

The total shown here in this table is 12, but I believe we have looked at 20 others in the Galveston Bay area. And, again, the highest found was about 1/10th of a part per million in the oysters.

We have looked at some miscellaneous foods.

One sample of cat food showed 6 parts per million of PCB.

A sample of crab meat showed 2/10ths of a part per million.

We even analyzed the coating that was used on one of these silos involved in the milk contamination, and the coating contained 11 parts per million.

We looked at a sample of wheat, and it had 3/10ths of a part per million, while one of straw had 3/10ths also.

These are a limited number of samples, from which I think it is impossible to draw any firm and reliable conclusions.

We have also, of course, begun to look for PCB in our total-diet studies.

In the fiscal year 1970, out of our 30 market baskets, we found one composite of dairy products positive for PCB. It contained 0.05 parts per million. In three market baskets we found the meat, fish, and poultry to contain PCB.

Again, the highest level was about a tenth of a part per million.

We also found one sugar composite positive, which includes candy, incidentally. And we think probably the PCB was in the candy, perhaps, in a wrapper or from some other source.

The level, again, was very low, 0.08 parts per million.

In 1971, with 19 market baskets reported thus far, the frequency has gone up in the case of the meat, fish, and poultry composites. We have had 11 positives, ranging again to 0.15 of a part per million.

Also, we have found two instances where the cereal products composite contained PCB. And in one instance this was traced back

to the shredded wheat in that composite, which was picking up PCB from the container that had been fabricated from waste paper.

More recently we have looked at some waste paper and have gotten some information from the paper industry. And it appears that waste paper generally has PCB in it ranging up to 700 parts per million.

The so-called carbonless carbon paper runs very high in PCB.

In other words, in summary, we have analyzed about 9,000 samples of food in the past year for PCB, and about 500 of those were positive.

DR. KOLBYE: Are there any questions directed to Mr. Ramsey?

I might add one note. Lea, I am not sure you mentioned this.

So far we have not reported the residue of PCB in fresh fruits and vegetables.

MR. RAMSEY: I should have said that we have not found it.

DR. BOOTH: I would like to ask a question regarding the PCB levels in shredded wheat and also in some of the other plant materials that were mentioned.

You did mention that it probably came from the wrapper in the case of shredded wheat. I was wondering if plants can take up PCBs.

We do know that, in the literature, it is indicated that plants do take up DDT.

DR. KOLBYE: I can't speak for that specific point. I don't know about the translocation of it. I would presume it can occur, but I am not competent to speak to that particular point.

Does anybody care to answer that?

DR. KEARNEY: We have looked for a number of years at residues in soils and translocation from soils into plants.

In the case of DDT, we find some movement of soil residues into plant materials. It depends on many, many things, as you can imagine, such as concentration, plant species, soil organic matter, temperature, and a myriad of other things.

What he have found recently in the case of DDT -- which chemically mimics PCBs in many ways -- that the closer the material is to the surface, the greater the contamination in the plant from volatility.

There is the possibility of PCB residues in soils giving rise to residues in plants, but depending on many environmental factors.

DR. KOLBYE: I might add that this recycling of paper products situation may present us with a long-term problem in PCBs with respect to the manufacture of chip board and things like this which can come into contact with food surfaces. It is one thing to eliminate PCB from the National Cash Register Company paper but apparently there is not for PCB in the paper that is already marketed and recycled so that these manufacturers cannot eliminate PCB entirely. So it looks like this is one of the more immediate questions the FDA has to face in attempting to regulate that exposure.

Bill Cook, do you have anything further that you would care to add on the chemistry?

MR. COOK: I have little to add but I thought I might address myself to a question that Dr. Jurger raised and two or three other points that might be of interest to those of you who may not be close to the laboratory.

You asked about specificity identity. For us in Food and Drug this is very important. As has been mentioned a number of times, the PCBs, 1254 and 1260 appear with the pesticides through the analytical procedure. When we do find PCB we separate them from pesticides. 1254 and 1260 are very easy to separate. We also have confirmation tests. These Aroclors are very stable to alkali, for instance, and we go through an alkali test.

Many of the pesticides such as DDT are easily altered by alkali. Besides that when there is a question one can use mass spectrometry. We don't do that routinely.

A number of people have spoken about quantities. I think it is important to recognize that there are some problems of quantitation, means of making calculations. Generally speaking, in fish the identity of PCB appears to be 1254 or 1260. Those chromatograms do not look exactly like the chromatograms obtained from standards. We make a comparison of all of the area under the curve from a standard against all of the area under the unknown curve using either the electron capture or for the microcoulmetric detectors.

We know of instances in which people make their calculation from two or three peaks. I know one individual who makes his calculations based on DDE as a reference. These different means of basing calcu-

lations will result in different values.

One point on that carbonless carbon paper, both the Los Angeles and Seattle FDA Districts, and we in the Washington laboratory have done some work on a few of those pieces of paper. The lowest value we found was one percent by weight and the highest, from a copy of the Internal Revenue Service, Form 1040ES was 4.5 percent by weight of PCB 1242. That is the chemical that was used by the National Cash Register Company in their carbonless carbon paper. They have their patent leased out to another company, Meade, and as far as we know now they too have discontinued the use of PCB in making carbonless carbon paper.

However, as Dr. Kolbye indicated, this is going to continue to be recycling for some time to come. Somebody might even throw an old government file into the wastepaper; this may contain forms containing PCB and thus continue to add PCB to the cycle.

We have not done any work on the penetration of PCBs into plants but I would guess it would penetrate into root vegetables such as carrots as is the case with dieldrin and there may be no measurable translocation upward into the aerial parts of the plants. We in our lab have done a little work on the effect of ultraviolet light. This is a rough treatment. We knock some of the chlorines off; 1254 appears to become lesser chlorinated. These results have yet given us any concern because generally speaking, everybody throughout the world is reporting in wildlife residues which look like 1254 and not residues with less chlorine like 1242.

If there are any questions I will try to answer them.

DR. MORRIS: I recall that during the early days of the Food Additive Amendment we received food additive petitions covering the use of polychlorinated aromatics as indirect food additives. Did these early petitions include Monsanto's PCBs, and if so, what was the result?

MR. COOK: There was a petition for one of the compounds to be used as a component of an adhesive on packaging tapes. This was a chlorinated terpheynl, not a biphenyl. Mr. Ramsey could probably answer that better than I.

MR. RAMSEY: Yes. It is allowed in a food packaging adhesive on the basis that there is no migration into the food.

MR. COOK: When we speak of aroclors we are speaking of a mixture of chlorinated biphenyls, a mixture of chlorinated biphenyls and terphenyls or chlorinated terphenyls. The 4400 series is a mixture of the terphenyls and biphenyls, and the 5400 series are the terphenyls. Those higher ones are solids that seem to be very inert. The lower series such as the 1221, the 21 percent chlorine is even water soluble. It is very fluid.

DR. HALEY: They also will produce chemical necrosis is they get into the skin, the terphenyls. So they are not innocuous.

MR. COOK: I am not familiar with the toxicology that was presented on the petition.

DR. KOLBYE: Are there any further requests to Mr. Cook?

I think the point about the terphenyls is that as far as we know there is no migration. This can be checked. I think it would be interesting if the Food and Drug Administration could declare the Form 1040 a hazard to health but I am not sure it would be appreciated by the IRS.

DR. KRAYBILL: Dr. Lindsay has indicated to you that the status reports that we prepared, there are two of them -- the last one appeared in December -- are now very much out of date. I believe with information presented this morning that we have been scooped and this is a good thing since such information is most illuminating.

Another thought that came to my mind was the similarities in other areas of work such as research on the dioxons and some of the pesticides. In dioxon toxicology we encountered the problem of chloracne in occupational exposure and this morning a PCB toxic response was mentioned that is hydropericardium.

One may have some contaminants here as one observes with the dioxins. The polychlorinated biphenyls contaminants occurring together with those from dioxins.

If one looks over the various reports -- and there is an excellent Swedish report as well as the FDA status reports -- and realizing what we heard today, there are some voids in our knowledge, areas that we should explore to enlighten us a little more on the safety or the toxicity of these particular compounds.

One thing we should identify are the primary constituents

of the residues of PCB. This is a big talk. Of the 1,200 series, 42% and 68% and 60% chlorine compounds and so forth, fish, perhaps, as a food would be a good item to survey in this identification.

It would be helpful to know what chemical or molecular species we are dealing with which would help us in making our assessment of toxicity more meaningful. As an environmental health scientist, one always thinks of the total stress. Accordingly, Dr. Worf mentioned here this morning that he is collecting air level data on PCBs. I sure would like to see this data. There is some PCB water data, perhaps on potable water supply. There may be some PCB levels determined in the water in the estuaries where the fish swim around. We should have such data. Mr. Ramsey previously reported the data on foods and food composites in the diet.

A statement was made about fruits and vegetables as to non occurrence of PCB. We have reason to feel that some of these classes of foods don't have the PCB residue, hence, we are talking largely about the meat, fish and poultry group, but that is one-fifth of daily poundage insofar as total intake of our diet. Another important aspect is that of human monitoring and Dr. Hammer and Mr. Burse talked about this.

The sort of information we definitely need is data on the body burden of these compounds. You did hear about the levels in the fat or adipose tissue, liver, kidney and brain relevant to the study in South Carolina. It is most interesting to have such data but I wonder perhaps if one should not acquire data from surveillance

across the country to see whether there are any geographic differences, EPA has the monitoring system to do this.

EPA has the resources available in about 15 community study projects. They represent an excellent geographic distribution.

The other thing one needs is some research on the effect of PCB on various cell systems. I think we should study the effect of these Aroclors on certain human cell cultures.

Insofar as toxicity is concerned, in short term toxicity the response that is most intriguing to me is the effect on the liver microsomal enzymes affected by the Aroclors. An effect on the liver microsomal enzymes by the Aroclors one might study farther, since this has been done to a certain degree. The influence of dilantin and hexobarbital, which interact with some of the pesticide compounds, have been explored such as DDT and dieldrin and lindane and compounds of that type as well as carbon tetrachloride. These have an important effect insofar as synergism in their interaction, therefore how much do they potentiate, which could have an analogous situation in real life.

Another area is the teratogenic study on the Aroclors with certain test systems, other than the chick embroyo. FDA has done extensive work on a wide series of Aroclors with the chick embryo and reported a host of biological defects from these various Aroclors.

Another area for study is the short term reproduction response, the effect on the fetus. This is most important. Last but not least -- there should be some work taking place on the mutagenic effect of these compounds. Then Mr. Burse and Dr. Hammer referred to

the metabolic studies and here we need a better idea perhaps, on the distribution of these Aroclors in the body, the metabolic rate, the retention times or turnover rates, and the excretion rate.

Also we should have a better picture on the fate of these compounds and their metabolites as it appears in the feces and urine. This brings to mind the identification of the Aroclor degradation products. Are the Aroclors broken down like DDT into metabolites -- such as DDE, DDA and DDD. In truth then what is the toxicity of these various metabolites?

Now we get into the long-term effects of these compounds and we immediately think of the carcinogenic properties of these compounds and we were interested to hear Dr. Kimbrough say she was first to note a few bladder tumors.

In this context, will these compounds attack other organ sites such as the kidney, the liver, the brain, et cetera.

Experiments in chickens and rodents do not follow a characteristic pattern insofar as reproduction. For example, the Aroclors seemed to be more toxic than DDT in these particular studies. I made an outline here that you may want to consider on what I called dissimilarities in PCB and DDT toxicity. We might cite some of these differences.

A. Apparently Aroclors have five times the activity that DDT has insofar as effect on the liver microsome enzymes. To me this is most important.

B. The reproduction experiments in the rodents and chickens which I alluded to, where I said the aroclors may appear to be more toxic than DDT.

C. The metabolites apparently are unknown as is their storage capabilities in the serum and body tissues.

D. The adipose tissue levels in man of Aroclors seems to be lower than that of DDT. At least, looking at the data of Dr. Hammer, he showed parts per billion levels in man in his surveillance studies.

E. The aroclors are not as universally distributed as DDT and this has been mentioned by several of the investigators. There is one other piece of information that caught my eye and that is in the test work with Aroclor 1221, 1248 and 1268, administered at a dose level of 50 parts per million. With Aroclor 1221 there was an 11 percent reduction in the sleep time. (hexabarbital used) With 1248, there is a 45-percent reduction of sleep time. With the 1268, there was a 48-percent reduction. So this would mean at increasing levels of chlorine content -- the reduction of sleep time goes up, again pointing up the importance of this interaction between a drug and a toxic compound, such as the Aroclors. Other data on toxicity is pretty well summarized in FDA status reports, and previous speakers here have given values on the LD-50, the effect on reproduction, etc.

DR. KOLBYE: Are there any questions directed to Dr. Krayhill?

DR. MORRIS: In light of the relatively small quantity of PCBs now being manufactured, it is difficult to understand how PCB has attained the same degree of criticality as DDT which has been manufactured in hundreds of thousands of tons, extending back over three decades. Do we really know how much PCB is now being produced?

Are there large sources other than Monsanto? The reported universal occurrence of residues indicates there may be sources which are currently unknown to us.

DR. KOLBYE: I don't think Monsanto has ever released data on what the volume of their previous production was.

DR. MORRIS: I understand we have made some pretty good estimates.

DR. KOLBYE: That may be. Who has the information to speak to that? I remember a meeting about a year ago in Dr. Burger's office where we couldn't get that information. Maybe it is available now.

Dr. Lindsay, that concludes our viewpoint, except perhaps for the suggestion that after the meeting today winds up and is concluded I would like to suggest that we have some sort of a continuing panel of representatives so that we can exchange information.

DR. KIMBROUGH: I would like to point out again that I do not want to make the statement that the PCBs are carcinogenic. However, we should set up studies to determine whether they are carcinogenic. I would also like to raise another question; reports have been made particularly in the older literature where people have developed chloracne after they have been exposed to PCBs. I wonder if chloracne has been observed recently? Do the workers who worked with the fishmeal show skin changes?

DR. KOLBYE: Does anyone have any information of that point? It might be interesting to check into the Japanese poisoning cases, too.

DR. NELSON: The Japanese do report that the poisoned show the colored individuals did have skin disorders.

DR. WORP: I would like to direct a question to Mr. Duggan. I would like to know whether the contaminated fishmeal is going to be disposed of in any regulated manner. What are the plans?

MR. DUGGAN: The contaminated fishmeal, of which there is now between 1,000 and 1,500 tons, is being collected at Wilmington. What will be done with it we don't know. This is the manufacturer's problem but it will not be used in food of feed. We will maintain control over it until it is properly disposed of, probably as fertilizer.

DR. KOLBYE: Fertilizer for non-food crops?

MR. DUGGAN: Are you going to enforce that?

DR. KOLBYE: No. I guess that is our hope.

MR. DUGGAN: On this fishmeal, in our initial investigation we did look for PCBs in the fishmeal coming off the boat and going into the pasteurizer. The results were negative. So it is not a general problem with fishmeal.

DR. LINDSAY: Henry Verhulst, would you report on the Bureau of Product Safety?

MR. VERHULST: I have very little to say because we are relatively new in this. We have the responsibility for all products going into the home, particularly those that are accessible to children. Recently, we have begun a project to investigate if PCBs are included in the plastics, particularly in children's articles. After today, undoubtedly, we will want to look into the matter of newsprint, dyes and otherproducts that are coming into the home.

Certainly all sources should be looked at as part of the total picture in terms of the problem that does exist.

DR. LINDSAY: I suspect we had better take a break for lunch. After lunch we would like to hear from other agencies that are here and from individuals who are working in the area of PCB research. Dr. Hoopingarner and others will be heard. Then we will come down to the crunch; where do we go from here?

As to lunch, I think the only advice I can give you is that if you went for coffee, that is also the nearest place to have lunch. Any place else is quite a little ways off, except there are several "blind" stands on different floors.

We will recess now and we will need to be back here, under these circumstances, by a quarter after one.

If there are no other questions, we will see you back here at a quarter after one.

(Whereupon, at 12:15 p.m., the hearing was recessed, to reconvene at 1:15 p.m., this same day.)

AFTERNOON SESSION

DR. LINDSAY: If we get started quickly, we won't be but more than the ten minutes or so late that we were before. That is pretty good.

Mr. Duggan, I think we have reached about the point where since I think you know the gentleman involved in other agencies better than I do, will you introduce any individual here from either Interior or NOAA, and ask them to give a situation report.

MR. DUGGAN: The last PCB meeting we had was held out at Patuxent, hosted by Dr. Dustman and Dr. Stickel. Dr. Dustman has been very much interested in this PCB problem since it first came to our attention. Dr. Dustman, would you speak to the point and have your group speak as they wish.

DR. DUSTMAN: Mr. Chairman, Mr. Duggan, ladies and gentlemen, we have been familiar with PCBs for a very long while. In the very early stages we didn't know what they were. The eagles told us that they were there. We learned very early in our chemical meanderings to stay shy of them, and developed methodology at Patuxent that allowed us to split them off, so to speak We did this by zoning, and it has served us well through the years.

I have with me from the Patuxent Wildlife Research Center, three people that I would like to acknowledge: Dr. Lucille Stickel, who is our environmental pollution research coordinator, and you will be hearing a little bit from her later on; Mr. Mulhern, who has recently published a method of separating suitably and measuring PCBs by use of a thin layer method, (this was published in JAOAC in May), I believe; and Mr. Heath, who is our Center biometrician, and also in charge of our toxicology work. He has carried on a number of tests, short term toxicity tests, using our standard test species, and also longer term tests, studying the effects of PCBs on reproduction.

We have as of last March a publication that is soon to make its appearance, entitled "The Occurrence and Significance of Polychlorinated Biphenyls in the Environment" that will be published in the Transactions of the North American Wildlife Conference that was held in Portland, Oregon.

With your permission, I would like to read a summary of this paper, some of which is well known to you; some I hope will be of such recent origin that it will be new knowledge. This paper is a review of the literature: there have been several other reviews mentioned here today. This one was written with particular emphasis on wildlife matters. It is some 24 or 25 pages in extent, and details those sorts of things that we are concerned with and engaged in in studies of environmental pollution as it relates to wild animals. There are several things here in the summary that I think you will find of interest.

I am sure you are all aware that foreign scientists have given a fair amount of attention to PCBs, and I would like to start by mentioning that special studies in the Netherlands, and Great Britain, as well as in the United States, have traced PCBs to industrial effluents, although other possible sources have not been followed. The use of PCBs in paints, cartons, and insulating fluids suggests that environmental pollution may be from many different sources.

PCBs are present in fish and wildlife in many countries of the world. Quantities are higher in animals living near industrial sites. PCBs build up in biological food chains with increases of tens to thousands of times from lower to higher organisms. This is of very great importance to those of us who are concerned with environmental effects.

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Experimental studies have shown that PCBs have a toxicity to mallards, pheasants, bobwhite quail, Coturnix quail, redwing blackbirds, starlings, cowbirds and grackles that is of the same order of toxicity as DDE to these species. Overt signs of poisoning also are similar to those caused by compounds of the DDT group. Toxic effects of DDE and Aroclor 1254 to Coturnix chicks were additive, but not synergistic.

PCBs containing higher percentages of chlorine are more toxic to birds than those containing lower percentages. And, of course, we have heard of the PCBs of foreign manufacture containing contaminants to an extent that greatly increased their toxicity.

So we have contaminants that are contaminating the environmental contaminants, if you want to put it that way.

Toxicity to insects of PCBs of different degrees of chlorination is the reverse of the pattern in birds. The lower chlorinations are more toxic to insects. PCBs enhance the toxicity of Dieldrin and DDT to insects.

Residues of PCBs in the brains of birds killed by these compounds measure in the hundreds of parts per million. PCBs may have contributed to mortality of some birds in the field.

PCBs induce microsomal enzyme activity in birds and mammals and the lower chlorinated mixtures have estrogenic activity in rats. This you have heard today earlier.

Offspring of pheasants whose parents received high dosages of PCBs made poor choices in visual cliff tests, indicating that behavior was affected. Egg production and hatching after pipping also were affected.

Long-term studies of the reproductive effects of Aroclor 1254 on mallards and bobwhite quail and of Aroclor 1254 plus DDE on quail showed no

significant differences from controls. This was work that Mr. Heath was responsible for at Patuxent. In studies of chickens, however, egg production, hatchability, and shell thickness were impaired by high doses of Aroclor 1254, and by low doses of Aroclor 1252.

With these few remarks, I would like to ask Dr. Stickel if she would come up and tell you a bit about what we have done, what we are doing, and what we plan to do at Patuxent in regard to studies of PCBs.

DR. STICKEL: Many of the principal findings Dr. Dustman has already covered. I think maybe the main point of our work that would be of concern to you is the demonstration of the universality of the occurrence of PCBs.

There has been some feeling that PCBs may be only a local problem, but from the evidence that we have at hand, the very great extent of the distribution of these materials in the natural world is becoming increasingly clear.

The PCBs have been present in substantial quantities in every bald eagle that we have analyzed at our Center from the time we got the first gas chromatograph. And the ones that have been collected and analyzed from the years 1967 through 1969, where it has been possible to get an approximation of the amounts, contain quantities of PCBs that are of the same order of magnitude as DDE, which means that the averages are in the tens of parts per million, and the extremes in the hundreds of parts per million.

There has been one case where PCBs probably were involved in the death of an eagle, with brain residues into the hundreds of parts per million. Ospreys have been sampled -- both tissues of birds and the eggs that they lay --

in a number of areas along the Atlantic Coast. Connecticut osprey eggs contained an average of 16 parts per million PCBs, with amounts ranging from 7 to 30 parts per million.

Eagle eggs had an average of 10 parts per million of PCBs and even in Alaska, 1.65 parts per million.

Ospreys eat nothing but fish and the fish haven't been through the processing plants to be directly contaminated by leaking heat exchanges. Eagles feed primarily on fish, but also eat some birds. So that they are working on the same food base that we are.

We have also made surveys of woodcock, a bird that is of considerable interest to hunters and that feeds altogether on earth worms. A woodcock eats an average of 75 percent of its weight a day in worms. Their tissues on a fat basis contained an average of four to nine parts per million of PCBs. The worms they feed upon are living in the same soils where we are growing our crops, and again, these birds are a product of the same food base that we depend upon.

We have sampled waterfowl nationwide; all samples have contained PCBs at various levels from low to high, depending upon the regions. The only birds that we have sampled that have not had PCBs in every sample are mourning doves. The breast meat of mourning doves, the Southern hunters will be pleased to know, are extremely low in all DDT products, PCBs, and mercury.

So, if you want to go hunting, don't hunt eagles; hunt mourning doves.

The really important part of our work, however, is not so much the

distribution of PCBs in the environment and the levels of residues that are encountered, but the significance of these residues. Mr. Heath is conducting research to determine the effects of the various formulations of PCBs on the reproduction of birds, and on their survival.

The PCBs follow the standard dose-response relationships that have been found for other toxic chemicals and toxicities have been established by probit analysis techniques.

The reproductive studies have not been conducted with all the different formulations. There are some signs of species differences in response to PCBs, which is also true for response to DDE. The species that are sensitive to these two materials may be somewhat different, however. For example, bobwhite quail and pheasants are birds that are not susceptible to DDE-induced shell thinning except in a very minor degree. Their shells can be thinned by massive doses.

But, whereas mallard ducks are sensitive to DDE, the opposite may well be true with PCBs. Mr. Heath's studies showed no reproductive effects of PCBs on mallard ducks at anything that would approach a realistic environmental level. In contrast, the Monsanto Chemical Company's studies with chickens did show shell thinning as a result of dosage with certain PCBs.

This has not been pursued sufficiently yet to be sure, because all the formulations have not been tested against ducks and chickens at the same dosages; the cross-comparisons are still an approximation and may not prove to be correct.

In addition to these studies, we have special studies in residue kinetics, which are studies of how to diagnose the cause of death due to PCBs, determination of the tissues to analyze, and the critical residue

levels. They also include studies of how rapidly the chemicals build up in the tissues and how rapidly the birds can rid their systems of the material. These studies are not very far along, so I can't give you any results at this point.

We also have a chemistry laboratory which of course is basic to the success of any of our enterprises. Some of our first results have been published, giving the mass spectra and empirical formulas of 18 different PCB materials in eagle tissues. Mr. Mulhern's work with thin layer chromatography has led to a method for quantification and separation of PCBs. Mr. Bagly has made studies of the metabolic changes of PCBs in quail and these results are nearly ready for publication.

An important problem in chemical analysis was brought out this morning. This was that the pattern of PCBs changes in the animal body, and keeps on changing with time, so that quantification from total areas, using the Aroclor mixtures as standards will leave something to be desired in terms of accuracy.

However, as nearly as we have been able to determine from the magnitudes that have been found in wild animal tissues by different people using different methods, it does not appear that different biological conclusions are going to be drawn. All measurments are roughly the same magnitudes; so that it's a matter of improving precision. Despite the variations in methodology, it does not appear that they have produced misleading conclusions in the biological sense.

I have been asked to call your attention to a publication, "Chlorinated Hydrocarbons in the Marine Environment," which is a National Academy of Sciences publication that includes a discussion of PCBs.

MR. DUGGAN: Since neither Doctors Dustman nor Stickel invited question, I think they would both be willing to answer any questions that you might have of them.

Are there any question?

DR. WORF: I was wondering what the sources of polychlorinated hydrocarbons are in the marine environment and is it possible that some of the sources might be from ships that have sunk that have used tons -- literally tons of PCBs in transformers?

I happen to be involved in the transformer plant operations a number of years ago and one of their main customers were ships that used transformers. Is this a possibility?

I shouldn't really ask you. I would suggest that as a possibility.

DR. STICKEL: There is a possibility; however, quite a bit of work is being done in England and they have managed to trace PCBs to the sewage outfalls from industrial plants. This material has been taken and dumped in the Clyde Estuary, and in certain other places around the coast. It would appear that the levels of contamination in their fish were sufficiently well explained by the amounts that were dumped.

DR. HAMMER: Dr. Hammer, EPA.

I just wanted to clarify, did you say that woodcocks had the 49 parts of the PCBs, or the earthworms?

DR. STICKEL: Four to nine.

DR. HAMMER: Is that in the woodcocks or in the worms?

DR. STICKEL: On the woodcock breast muscle.

DR. HAMMER: What was in the earthworms, do you know?

DR. STICKEL: No.

DR. LINDSAY: Are there any other questions?

If not, we have Mr. Tom Carver, and Dr. Emerson, and I believe someone else from the National Marine Fisheries.

Tom, would you care to make a few remarks or would some of your colleagues make a few remarks?

MR. CARVER: I can do it from right here. Reo asked us to come as observers, and we can best do that by sitting down. The Service rather abruptly terminated its PCB research program when Tom Dukes laboratory was transferred to EPA. There is one other aspect that I should mention.

The estuarian portion of the national pesticide monitoring program has been looking for PCBs in shellfish at about 170 locations around the country. This program is also conducted at Tom's laboratory in Gulf Breeze.

The results in general you have heard earlier from Food and Drug. We have found PCBs in oysters at very low levels, and very infrequently, and I think most of these came from Southern waters. I believe most of the elements of the national pesticide monitoring program have included PCBs in their analyses.

Is this essentially correct?

MR. DUGGAN: Particularly in the past year.

MR. CARVER: Good.

An the results of these studies will be printed in the Pesticide Monitoring Journal on a continuing basis, so maybe we can sell a few more copies of that.

DR. LINDSAY: Are there any questions of Mr. Carver?

If not, thank you, Mr. Carver. Thank you Reo.

That brings us down to people who are doing research. As I indicated earlier this morning, we asked agencies as we contacted them if they wished

to bring in someone from outside the agency that was either working under their funding or about whom they know and what they thought should be here.

We brought two people in. Their names both begin with "H" so, if I go alphabetically, I guess I have to go to the second letter -- in which case that puts Dr. Harris in front of Dr. Hoopingarner. Dr. Harris, from North Carolina State University, was one of the people that we were looking to quite early in the episode of the Holly Farms chickens.

Dr. Harris, could you tell us a little about that -- and anything else?

DR. HARRIS: Thank you Mr. Chairman.

We became interested in Aroclor when the inspection service began to pick up residues in poultry meat, after the New York fiasco. We had some tests underway to determine the toxic effects on poultry.

On June the 9th, 1971, a condition in two-week old broiler chickens with symptoms similar to chick edema disease as described by Schmittle and Sanger was seen.

The most common symptoms were gasping and incoordination of the head and neck just prior to death. The most severely affected birds emitted a squeaking sound. The mortality ran to 50 percent and the remainder were stunted to the point that they were destroyed. The condition occurred in several broiler flocks from one day's feed production and no further cases developed.

On postmortem examination, most birds had a severe hydropericardium with sufficient fluid in the peritoneal cavity to cause distention. A greenish yellow gelatinous-like fluid was found under the skin of some of the birds. The livers showed varying degrees of degeneration, with a mottled to a roughened appearance resembling cirrhosis. The kidneys were swollen

and pale with small hemorrhages. Some of the birds had white caseous-like material, resembling a bacterial infection over the heart and the liver. There was inflammation of the intestinal tract but no extensive enteritis. The digestive tract was usually empty.

Becuase of the sporadic nature of the condition and the resemblance of chick edema disease or crotalaria poisoning, a toxic substance in the feed was suspected. Feed from houses with affected chickens was collected for analysis and a feeding experiment.

Forty one-day old broiler chicks from the same parent flock were equally divided and placed in a battery brooder. Twenty were given free access to the suspect feed and twenty to a known non-toxic feed that is routinely fed in the Department. The chicks were observed daily as to appearance, feed and water consumption. At ten days of age, the chicks on the suspect feed were not very active and would huddle close to the source of heat. Feathers were ruffled and some of the birds were gasping. At fourteen days, half of the birds on suspect feed had stopped eating and would not move about in their cages. On the fifteenth day, ten birds died and the remainder were showing symptoms of gasping. The twenty birds on control feed were normal. All the birds on suspect feed were autopsied and had typical lesions, as we had observed in the previous birds dying in the broiler houses. In some of the birds, we were able to withdraw 45 ml of fluid from their pericardial sac and peritoneal cavity.

A sample of the suspect feed was analyzed for chick edema factor, chlorinated hydrocarbon pesticides, crotalaria seed and polychlorinated biphenyls as McCune and Flick have described the latter as producing symptoms and lesions as we had observed. The North Carolina Department of

Agriculture's seed laboratory found no crotalaria seed.

Analysis of the suspect feed contained DDE, 1.3 ppm; DDD, 1.42 parts per million; PCB, 148 parts per million; Benzene Hexachloride, 8.8 parts per million, Chick Edema Factor, negative.

The principal toxic ingredient in the feed was considered to be PCB. The only common denominator we had was fishmeal coming from the Port of Wilmington. We alerted the feed company as to the toxic effects they were having with their feed and the cause. We then notified the Department of Agriculture, who has this in their jurisdiction.

The gross pathology has been described in the literature by McCune, and we feel that based on his work, there is a synergistic effect between the benzene hexachloride we found and PCB.

The main problem experienced by the poultry industry was the effects on hatchability. Breeder flocks that produce the broiler chickens previously were hatching 88 percent and some went down to as low as five percent.

We did some analysis of PCB on chicken embryos. These were 14 one-day old dead embryos. They contained .35 parts per million PCB. The yolk of fertile eggs contained .97 parts per million, and the fresh yolk material from eggs prior to being placed in the incubator contained .29 parts per million. These were all from breeder flocks that experienced lowered hatchability.

We are doing the histopathology on the sections collected from these birds, and we'll have reports on this at a later date. I will be glad to entertain any questions that you might have regarding the problems we have in North Carolina.

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DR. LINDSAY: Thank you, Dr. Harris. I think as Dr. Mercer observed earlier this morning, it is hard for a group of people getting up sequentially this way to leave anything for anyone else to say.

Dr. Hoopingarner from Michigan State University is a member of the team that is working on the problem of mid-west meat kill, and I'll leave it up to him to describe more of their efforts.

Dr. Hoopingarner.

DR. HOOPINGARNER: Thank you, Dr. Lindsay.

I come here by default. Dr. Ringer and Dr. Johnson probably should be here, but since they are on vacation, I won the toss.

Michigan comes by this problem in a long series of events much like you have described. It starts back essentially with the opening of the St. Lawrence Seaway or before, when the alewife moved into the Great Lakes. This is a little rough fish, not too important, except when they die by senescence, and they pile up in great numbers on the beaches of Lake Michigan and Lake Superior.

To combat this, the Michigan Department of Natural Resources decided to import some game fish, since they had essentially controlled the lamprey, which had been destroying much of the lake trout. These game fish made tremendous gains. Probably some of the greatest weight gains in recorded fish history -- at least so I am told.

The first subsequent generation of coho, that is the eggs and young hatchlings, died in great numbers, in what apparently was DDT poisoning. That is, the people in fisheries, Dr. Howard Johnson, said that this appeared to be what was happening. So they undertook an extensive program in monitoring of the mortality of the young fish.

Of course, prior to this time they had also monitored the DDT or chlorinated pesticides within these fish and had found quite large quantities of DDT. In fact, so large that most of the fish were not allowed to be shipped inter-state.

In the subsequent years since the first harvest of the fish, the young were still dying, but now the symptomology had changed. This always looks like Monday morning quarterbacking, but essentially there was a confusion in the symptomology.

This is when they started with the problem of looking for other possible contaminants: mercury, TFM, many of these became suspect, along with PCBs. The work is still continuing.

About the same time, since the coho come to the wiers in tremendous numbers -- and they have virtually tons of the fish available for the asking - and so they were fed to mink, primarily in Wisconsin, but some in Michigan.

These mink fed the fish product had few young, and the young that they did produce almost all died.

They attributed this to DDT poisoning or chlorinated pesticides and they stopped feeding coho.

A year ago, Dr. Ringer at the University decided to test just that hypothesis, and he fed pesticides to mink in very high quantities, higher than found in coho and he found no apparent reduction in reproduction in mink.

They had a few mink left over, and so they decided to try some PCBs. The analysis of coho showed about 15 parts per million PCB. So they decided to double that level. They used ten parts per million each of 1242,

1254 and 1260, and fed it to mink. They didn't fail to reproduce; they failed to live. This is where the research essentially is currently at Michigan State.

They are now beginning a new season feeding kits and leading up to a reproductive season, and we have initiated a cooperative program with four of us trying to analyze some of the problems. The levels of PCBs will be much lower so that they can inhibit, they hope, reproduction in the mink. But certainly it appears now that mink are very, very susceptible to PCBs.

While I'm at it, I might say what the rest of us are working on at Michigan State. Dr. Howard Johnson is concerned with the reproduction in fish (coho, and other game fish) in Lake Michigan. Dr. Matt Zabik is primarily interested in the analytical chemistry aspects, but also in photo decomposition. My own personal interests are in the cytotoxicity to various cell cultures. We have a number of mammalian and insect cell cultures, and also interested in the so-called mutagenic effects, chromosomal effects, if you wish, to these same cell cultures.

I'll try to answer questions as much as I am able.

DR. KOLBYE: Dr. Kolbye.

Have you observed any chromosomal defects?

DR. HOOPINGARNER: The program is just underway.

DR MERCER: How long were the mink on the 30 parts per million before you saw the mortalities?

DR. HOOPINGARNER: I will have to beg off this a little bit as I may be wrong, but it was a very short time -- like 60 days, and they were dead -- before they could reproduce.

DR. KIMBROUGH: Renati Kimbrough from Atlanta.

You did not observe any porphyria in these mink by any chance?

DR. HOOPINGARNER: They have most of the tissues fixed, sectioned, stained, and are reading the slides at this time. I can't say for sure what pathalogical symptoms occurred prior to death as I don't know. And the fact is, I think, to be honest with you, the death took them quite by surprise. They expected some inhibition of reproduction, but not death.

DR. SENTI: What was the ppm of the feed?

DR. HOOPINGARNER: 10 ppm each of 1242, 1254 and 1260.

DR. LINDSAY: Thank you. Are there any other investigators in the audience who would like to discuss their research, and particularly any part of that research that bears on the part of the problems of the agency represented here today?

If not, then we come down to the hard part: it has already been suggested this morning that there should be a follow up of this particular type of information exchange. At lunch I discussed with Dr. Burger, the possibility that the Office of Science and Technology or the Office of the Council on Environmental Quality might act as an oversight agency to provide coordination for this type of information. Such a sponsor might follow this meeting with a more definitive meeting that could be structured and divided into areas where a more productive and profitable exchange of technical details could be given.

However, our exchange today leaves us with at least four areas of inquiry. I think perhaps the first of these has been fairly well answered and I don't know that we need to try to recap too much. From the material given today, what do we know about the Aroclors, or PCBs; but I don't mean to preclude anyone from speaking to that point.

A second one is obviously: what don't we know that we should know?

A third item then, of course, is how do you put these in priorities?

Lastly, and I don't know whether anybody today will want to speak to it or not, there is the area of needed legislation. I think that the Environmental Protection Agency may have some discussion they may want to undertake with regard to legislative hearings that have just been held. I know that Congressman Ryan of New York has been very anxious to get rather sternly repressive measures on the PCBs.

But I would like to throw this open to discussion. I think perhaps just to begin with, if Dr. Dick Gordon as openers could outline some of the questions that need answering, and knowledge that needs to be gained. After him, I certainly would like to have volunteers.

Dr. Gordon.

DR. GORDON: It seems to me that there are three areas of inquiry: What don't we know that we should? How do we establish investigative priorities? And, what are possible legislative and regulatory remedies?

I thought of five "D's" and a "B" -- Danger, Degradation, Disposition, Disclosure, Discontinuance, and a thought on Behavior.

I think the first question we might want to discuss is do we have a clear and present danger, or do we only have a potential danger? The mink experiment, for example, might be one of the few examples of acute mammalian toxicity reported for these materials.

From the point of view of degradation, the PCBs were designed to resist oxidation under elevated temperatures, last much longer than the DDTs, et cetera. Therefore, the environmental degradation pathways need much greater elucidation.

I think another interesting question that came up today concerns disposition. What about the fishmeal? In general, what shall we do with contaminated material? How shall we dispose of it? For example, if we put the fishmeal in the ground, from what we know we will not get rid of the PCB. If we put the fishmeal into ordinary incinerators, it is unlikely that the PCB will do more than be volatilized into the air. So the whole disposition of contaminated material is very much a problem.

The problem of disclosure -- how much has been made, where is it being used, and particularly how much is being made overseas, how much is being imported into the U.S.? Are other countries becoming alarmed? And so on? I think Ed Berger had some good thoughts that should be discussed further.

There is the Draconian step of discontinuance. Monsanto, as I understand did just that in England. Whether they should be so directed or requested to do so in the United States should be resolved. In any case we have a residual problem of no mean magnitude.

The last point came from some of the wildlife studies. This work has impressed me over the years: because one of the great things which the wildlife people have done is carefully observe animal behavior; as a result they are able to note small changes.

We do not usually get from the ordinary laboratory animal experimentation observations concerning long term changes in behavior particularly changes that might be very minor. Since the United States' population is addicted to one thing or another, nicotine or cafein and all these other things, the question of human behavioral change is one I think that Food and Drug and EPA should plan long term experiments on.

We really want to think more about this. Man is a social animal and reports dealing with minor changes in behavior patterns should be followed up. We all know that small residues in nervous systems can sometimes have very profound consequences.

By way of summary, I made these points which might allow us to focus the discussion we heard today.

Do we have a danger; what about the degradation of the PCBs? How do we dispose of contaminated material? What kinds of additional disclosures, as well as exchange of information do we need? And what kind of prohibitions shall be requested or promulgated? Finally, can behavioral affects be used as very sensitive indicators?

DR. LINDSAY: Any questions of Dr. Gordon? He was asking a question of everybody before he sat down.

Dr. Kraybill, of course, had already summarized in quite succinct fashion many of the problems that are left unanswered by the knowledge that we have, and he has had admirable insight into this as the compiler of the status reports that we have had.

And I would invite him, if he has any further items to add, to please do so. Do you have any comments beyond those you made earlier this morning, Herman? Or some you would like to make over?

DR. KRAYBILL: We might comment further -- and I mentioned this morning -- the similarity in the chemical structures, to the dioxins. We know from work in our laboratory the way the chlorine atoms are arranged around the nucleus; if you get them all arranged around one benzene nucleus this compound is not as toxic. So here one might consider the isomeric form of these compounds and I am sure that through our chemical studies we'll be able to identify what specific chemical entity or what molecular species we are dealing with.

Well, this eventually takes us to a point in studying molecular configuration versus toxicity. We know a lot about the dioxin toxicity, and this would be very helpful, comparatively so in identifying the various molecular species and then assessing the actual toxicity of that particular molecular entity.

DR. LINDSAY: When we wound up the Bureau of Foods' presentations, I neglected to call on the Bureau of Drugs.

We have several representatives here. I just wondered if there were any comments they might have to make other than the fact that, "Gee, aren't we glad we don't have to mess with this."

Dr. D'Aguanno?

DR. D'AGUANNO: We live in a drug oriented culture. The Bureau of Drugs has been primarily a drug oriented culture. We have been aware of interactions. We are barely touching drug interactions, outside of an occasional report in the literature on drug pesticide interactions in humans -- I guess these were animal studies, laboratory studies. This is the extent of what we have gone beyond drugs.

DR. LINDSAY: Well, we trust you'll keep in touch with all of this now as it goes along.

Dr. Thomas Haley is with us in the back of the room, and any fellow that has done as much pharmacological work as he has done has some comments, I am sure.

DR. HALEY: I'm Haley.

Yes, there's two things that I heard today that I think should be followed up.

First of all the sex difference. I didn't hear anything about that.

DR. LINDSAY: Turn that up a little -- I think you said "sex"?

DR. HALEY: Yes -- the difference between the male and the female --

(Laughter.)

DR. HALEY: I believe there should be studies on castrates, and the use of hormones to see if you can restore the difference.

Secondly, the decreased hematocrit and the decreased red cell count and the deposition of porphyria, means that the Δ- aminolevulinic acid cycle in the bone marrow is being effected. This may be effected primarily in the same way that lead does it. I think the ALA synthesis should be studied in the presence of these compounds. It might give you a lead as to why this is coming about.

DR. LINDSAY: Thank you, Dr. Haley.

Dr. Flick?

DR. FLICK: I think I can speak loud enough.

We have found with the dioxin problem that the level of dietary fat played a role in the toxic responses of chicks, that is when the level of dietary fat was high, we did not get as much of a toxic response as when we lowered the fat content, which meant that we were facilitating lipid absorption and lipid component absorption, perhaps all lipid-soluble components.

On the other hand, when we added dietary bilesalt, this facilitated absorption at both levels of dietary fat. And I would suspect that perhaps the absorbability of the polychlorinated biphenyls could be facilitated in a similar manner.

Dr. Harris mentioned a while ago that the intestinal contents of the chicks observed in the Carolina outbreaks were essentially empty. We found this to be true not only with the dioxin studies but also with the polychlorinated biphenyl studies.

And in both studies we found in the terminal stages, that the animals' intestinal contents were essentially nil, and we never printed, but we've often discussed and still feel, that these terminal animals are really dying in a stage of malnutrition -- in addition to other toxic responses at the enzyme or subcellular particulate level.

Again perhaps dietary composition should be looked into with respect to the polychlorinated biphenyls. With respect to absorbability, we found this to be true, in a recent report we gave to AOAC last fall, that the absorbability of the dioxins were related to chlorine content. Again this may be a factor to be studied with respect to PCBs.

DR. LINDSAY: Thank you, Dr. Flick.

I think in getting down my list of draftees, it might be an appropriate time, Dr. Burger, if you have any thoughts with regard to what went on here today, and what might succeed it?

DR. BURGER: Dr. Lindsay, first of all, thank you very much. Let me just take the opportunity of offering my congratualtions to you for -- and the Food and Drug Administration for having put on this meeting, which I think is a landmark of a success in bringing the right variety of people together at the right time on this important subject.

And I quite sincerely mean that.

Our view of this, from my end of town, is perhaps several-fold. On the one hand, one could say this is a fascinating if academic case study of a scientific matter that impinges upon the public policy machinery. I

believe, incidentally that our place in life, as someone described it, is the marriage or estrangement between the scientific community and the public policy making machinery.

But think that indeed this is representative of a matter which is at the crossroacs of some public policy decisions. Let me just explore a couple of these with you, because I think they are terribly important. It seems to me that if one were to summarize much of what was spelled out this morning, and partly this afternoon, it deals with the probability of exposure of humans and of non-human elements in the environment. The amount of PCB material produced and its distribution in the man's surroundings and the rest of the environment, the possibility of second order interactions such as magnification or changes in chemical composition, and following from that, the biological availability to various species in the environment.

I think these are all matters which illustrate in part the complexity of the question, but also illustrate in part the fact that some of what we are talking about can be reduced to what might be called an information question.

There is a second kind of information question that has been addressed here. This is, given a figure of exposure to elements of the environment or a human being, what is the inherent biological hazard or activity that is involved? Perhaps from those two kinds of information, one then is in a better position to make some social judgments about whether or not this chemical material is a hazard or a matter of concern.

Dick Gordon raised the proper point, I think, when he questioned whether PCB is an established hazard or an implied hazard. This, of course, is illustrative of really a long list of materials that are now in man's

environment.

This then raises a series of additional points such as the responsibilities of the private and public sectors to do the research from which information is used to make decisions, the question of responsibility of divulging information about production and distribution and toxicity, unexpected side effects, and so forth. And thirdly, (indeed the questions become more difficult I suppose the farther I go down this list) there is the matter of the responsibilities of the public and private sector for controlling the distribution and regulating the production of a material which is either implied or known to be of some concern or even hazardous.

If I were to back up at this point, again, I would guess I am terribly impressed that much of what we have talked about can be called an information problem, and that having in hand the information about the amount of material that we are talking about and the ways in which it is distributed, aids enormously in making some decisions about how we view it.

Dale, you raised the question of legislation. I feel there is a piece of legislation now pending. The Toxic Substances Bill indeed was written in part I think to address some of these very questions.

Fourthly, I guess, I would raise the matter of candor. Although the subject of this conference represents an area where there is a history perhaps of less candor than some would desire, I am impressed that this government does its business with greater public participation than many others around the world. I think I sense that the government is expressing more candor than it has perhaps in several years. I think this meeting in fact is an illustration of that trend. Finally, in order to be able to deal in a candid way with subjects like this, obviously the government is

in a better position, the better armed it is with good science to back its decisions.

Again, this meeting has drawn out of the walls I think an enormous amount of effort and a great deal of diversity which has been terribly useful. It is really, I suppose, most frequently with this last subject, the quality of the science that goes into government decisions, that our office is most concerned.

Let me say quite frankly that, Dale, we would be very happy in cooperation with CEQ if they would like, to be able to continue this session in depth and at length.

Thank you very much.

DR. LINDSAY: Any question of Dr. Burger?

We have covered now the executive sector. Let us go to the legislative sector, I mean, from the oversight agency area.

Dr. Muir would you care to say something?

DR. MUIR: Thank you, Dr. Lindsay.

CEQ is an environmental overview agency of rather new origin.

Over the past year the Council has attempted to evaluate the many various environmental problems. Well aware of the problem of hazardous materials, including PCBs, we undertook a rather detailed study. The result was our Toxic Substances Report which was published this spring. If anybody is not familiar with the report more copies are available at the office.

A second product of this study was the development of the Toxic Substances Control Act of 1971, now pending in Congress, for which the Senate Commerce Committee's Subcommittee on Environment has heard testimony ove the past three days, in the first of two sets of hearing.

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Basically what I want to do is just quickly outline some of the provisions in the Act to show what is envisioned as a possible mode for attacking the PCB problem, and problems associated with the many other toxic substances which have been and will be introduced into our surroundings.

The Toxic Substances Control Act provides for the Administrator of the Environmental Protection Agency to regulate the use and distribution of all chemical substances. It further provides for the establishment of test protocols and testing standards for all new chemicals being produced in commercial quantities.

As Dr. Burger has indicated, one of the real needs in this area is information dissemination. The bill provides for full disclosure of manufacturing data with appropriate provisions for proprietary information. It will create a chemical compound classification system, and information storage system, to handle the kind of information that we have brought together today.

As some of the speakers have mentioned today, there exists similarities between dioxons and PCBs that should be noted. It is possible analysis of these chemical similarities could lead us to suspect other classes or other types of compounds that might cause environmental hazards. This classification-information storage system will serve to make these correlations and will attempt to provide for technological assessment and the prediction of developing technologies and new chemicals. Through this provision, therefore, it is hoped that we will be in a better position to prevent adverse effects from new chemicals prior to their release into the environment in large quantities.

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Finally, the bill contains a section intended to considerably expand research in the area of toxic substances.

Now this problem is obviously very complex. Any new program in this area will necessarily have to overcome difficulties. There will have to be careful coordination of the existing authorities and agencies currently concerned with toxic substances. Further, careful consideration will have to be made to decide the appropriate point for regulation of use in the sequence from mining to retailing of foods. At the very least, this legislation is a good first step in the direction required to tackle the problem of PCBs and other hazardous chemical substances.

I will be glad to answer any questions that anyone would like to raise about this legislation.

DR. LINDSAY: Any question of Dr. Muir before he gets down?

Dr. Fishbein, would you have any comments from a sister agency at HEW?

DR. FISHBEIN: No. We haven't been involved, actually, other than scratching the surface on the development of the analytical methodology of chlorinated and other contaminants of the PCBs. That is the extent of our involvement.

DR. LINDSAY: For those of you who couldn't hear, Dr. Fishbein said regretably that they have just been scratching the surface. I think that implies they are going to dig a little deeper.

DR. FISHBEIN: Yes, it does.

DR. LINDSAY: Well, now, I think we have come down to the gentleman that I want to call on next. I am always a little bit puzzled about just how to introduce him, because he has been so many places and does so many things.

Dr. Norton Nelson at one time, not too far back, was chairman of the FDA "Protocols for Safety Evaluation Committee." He is now part of the EPA Hazardous Materials Advisory Committee.

He has just retired as a member of the NIEHS Council. The main purpose in recounting these activities is that they all deal with the kind of problems we are discussing today, and he has been so involved for as long as I can remember. I think that Dr. Nelson, if he will try a wrap-up here, and if he follows his usual pattern of stimulating a lot of questions can effectively conclude our discussion. I hope that those of you who don't have airplanes to make at 4 o'clock or some such time can stay and ask your questions, because I think that Norton will probably give us a good deal that we can continue to discuss. He will be the last individual that I will call on.

So, Norton, if you will have at it, we would love to hear what you have to say.

DR. NELSON: Thank you, Dale.

Dr. Lindsay has opened up prospects which I am not prepared to fulfill. I really think that nearly everything that needs to have been said has been said and I see no reason to unduly prolong the discussion.

On the other hand, I perhaps perceive -- I am not sure I am right here or wholly fair -- a sort of midafternoon complacency descending over the assembled group.

A compliment was given to Dr. Lindsay for his energy in putting this meeting together. I join this tribute. This meeting signals the importance of the problem. Those who have spoken have presented some extremely important information, and a considerable part of it is new. Perhaps that sense of accomplishment drawn from a job well done may tempt us to sit back and relax.

Realistically, however, I do see a number of very great big gaps in this facing us in this problem. Thus, if I have anything to say perhaps I should attempt to point out the magnitude and significance of some of these gaps.

For example, we do not know the total production or use of PCBs in this country. Someone here may know this, I do not. These are vital data. Someone here may know how much foreign PCB is imported into this country, and used as such. Obviously we get some through finished products, plastics, et cetera. How much do we import as PCBs?

I did not see in any of the presentation today an ordered description of patterns of usage, distribution, turnover and loss which might permit us to determine the chief environmental sources of PCBs.

It is true that in several of the episodes the difficulty stemmed from accidents - thus, the misuse as herbicide in West Virginia, the leaky pasturizing equipment in the fish processing, the defective meat transfer units that produced the Yusho disease in Japan. These might give us the reassurance that we are dealing only with such accidents.

I suspect this is not the case. These accidents have served the very useful purpose of alerting us, sometimes belatedly, to the problem by producing easily discernible consequesces. However, I suspect that the wide spread environmental contamination may arise from continuing losses perhaps contributed to a series of accidental losses.

This could be wrong; however, I suggest that we have no reason for feeling any confidence as to what the sources of PCBs to the environment are.

Again, I believe we have only very crude ideas as to the patterns of movement of the PCBs from place to place, through the atmosphere and waterways.

We really do not yet have good data on the stability of PCBs in the various transfer media, sediments, et cetera. I see this as a very important consideration, not alone as it relates to future discharge but also with respect to the persistence of present contamination now found in many places throughout the world.

I am not going to speak of legislation. This has been touched on by Dr. Berger and Dr. Muir. I would say, however, that in our review of the sources of contamination, it may be determined that new regulations will be needed for control.

Again, we have talked about means of disposal of the PCBs or PCB containing materials. I suspect that there are not more than three or four incinerators in this country that are capable of successfully disposing of PCBs. In fact, I would suspect that most incinerators simply take it out of what may be a sequestered location and distil it into the environment where it is easily moved elsewhere.

There are questions relating to the biological effects of the PCBs; a few are fairly obvious. It is a remarkable circumstance, and if I am wrong please correct me, that the present interim guidelines, which the FDA has established, were set without benefit of a two-year chronic toxicity study of the usual, and indeed routine, type much less the fuller study that one would like to see with materials of this kind. Thus, I am not aware of any carefully done, chronic toxicity study that has been completed and analyzed and made available.

I believe that there are several chronic studies either near completion or perhaps recently completed. These will be very helpful when they become available. It is very clear that we urgently need carefully done chronic studies to answer the question as to whether malignancy does or does not occur.

We do have some data on the reproductive aspects, but this seems at best preliminary, rather than definitive. Indeed the fragmentary state of our knowledge of the toxicity of the PCBs is emphasized by the work at Michigan State on the minks. That work is compatible with the concept that there is at least an order of magnitude difference in the toxicity of PCBs different to mammals.

We have had discussion of the relative toxicity of the various isomers of PCBs. Although I suspect that the gross trends we now perceive will be sustained by more refined work, my confidence doesn't carry me to the point where I feel we can rest on this kind of speculation. We need far more detailed study of the various isomers.

Equally important is the question of contaminants or breakdown products; the chlorinated dibenzofurans have been very inadequately studied.

I have some other defects listed in my notes but these are the ones I see as being of major consequence. I hope that appropriate federal units will assume the responsibility for orderly steps to fill these and other gaps.

Thank you, Dale.

DR. LINDSAY: Thank you, Dr. Nelson.

Are there any other contributions that the afternoon somnolence will let you make?

Dr. Harris?

DR. HARRIS: There is some evidence, I believe, for certainly particulate explosion in the cities and this would fall out into the dust and dirt. I don't know if it would get into the food chain.

DR. LINDSAY: Dr. Kolbye?

DR. KOLBYE: I just would like to make a plea that somehow or another, we organize ourselves such that the flow of pertinent information concerning toxicology and residues is directed in such a way that the people that need to know this type of information get it quickly and can have it as a factor in their decision-making. I don't know what mechanism to suggest on this, but I would like to have it considered at this meeting, please.

DR. LINDSAY: Any response to Dr. Kolbye's question right now?

Dr. Dustman?

DR. DUSTMAN: I think it was about two years ago, if my memory serves me correctly, that a small group of individuals met in Duluth, Minnesota, for the purpose of exploring where we stood with PCBs and what we knew about them. The meeting was called by Dr. Don Mount of the National Water Quality Laboratory, now a member of EPA, and it started out to be just a few individuals who were interested that were going to get together for the purpose of seeing what we knew. And it grew into a full-blown meeting at which time quite a few university people were represented and a number of government people. I don't see many here that were in attendance at that meeting.

The important part of my comment relates to the development of a newsletter that was at that time agreed upon that would be issued at irregular intervals as the attainment of new information would dictate. And I have before me one such newsletter.

There have been three of these. They cover all aspects of the PCB business. Dr. Mount and his coworkers, there at the laboratory, have taken it upon themselves to issue this newsletter. It is one to which you should all subscribe if you wish to keep current on what is going on in this business. It covers such things as analytical methods, environmental occurrence and fate, uptake, retention, other physiological effects, and toxicity. The most recent issue is rather a thick one. There are many contributors to this. It is a very fine means of keeping up with what is going on and I would make the request of you that you really tune in on this and give encouragement to Dr. Mount who has, on his own, taken this on as an assignment. His address is: Environmental Protection Agency, National Water Quality Water Laboratory, 6201 Congdon Boulevard, Duluth, Minnesota 55804

DR. LINDSAY: Dr. Kolybe?

DR. KOLBYE: I think that is an excellent suggestion, and I would like to ask whether or not the resources of the National Library of Medicine, Toxicology Information Program, can be placed behind this type of an effort as an urgent priority matter.

DR. LINDSAY: I don't know. I had invited Dr. Mider here today but he had other priorities and wasn't able to come. I can't speak for the NLM but I would think that Dr. Kissman's group in the Toxicology Information Program would be very much interested in participating.

I would like to say in reference to your question just before, that I think a more proper way to get this interchange established, at least between the analyses that are involved here, would be for each of you people going back to your agencies to alert your agencies to this need,

particularly if you believe in it, and then some effort may be made on the part of the individuals for whom each of us work as to some decision regarding such a committee.

For example, something similar to the committee set up between Interior, Agriculture, and HEW with regard to the nitrite program. Dr. Leo Friedman is chairing that and he is not here to report on it. But that sort of thing I think is very valuable, and something like that could come out of this, Al, if that is what you had in mind. But I think probably we have the wrong people here to designate the individuals although we may feel to the contrary.

VOICE: I think we need both, a committee to coordinate among the agency resources and I think you need a superb toxicology and residue information program that can be disseminated broadly.

DR. LINDSAY: No argument.

Dr. Worf?

DR. WORF: I would like to ask a question either of Dr. Nelson or Doug over here, whether there is any occupation or health data? I alluded earlier that I used to work in an Allis-Chalmers plant that literally had thousands of tons of PCBs in the transformers, which I worked on in the laboratory, as a control chemist there, and I just wondered if there is any possibility of getting data from occupational health. I am sure that NCR, National Cash Register, their use of PCBs in paper and so forth, there is a lot of possibility of data from these sources.

Do you want to comment on that, Dr. Nelson? Is this reasonable?

DR. NELSON: I am afraid I cannot really help you. There is earlier history materials probably similar to these during World War II. I am not aware of more recent work which would relate to more modern patterns. They are probably very poor.

Maybe Dr. Hammer can respond to this. My feeling is that there may be data there, but I do not have it and I cannot say one way or the other.

DR. HAMMER: Just to underscore that, when I was reviewing this problem for our branch last year, the occupational health data was very scanty except for the problem of skin disorders and the problem of liver, acute liver diseases, et cetera. There was virtually nothing available from the occupational health literature, and I think the problem that those of us that are concerned with human health problems are having now with many of the toxic substances is that the kinds of exposures one saw in industry were really often very high, and they are useful for the acute clinical and sometimes chronic illnesses, but are not very useful at the other end of the spectrum, a problem of picking up burdens and going from there.

What do these PCB burdens mean? What is their long-term effect? We don't really know at present.

But I found just a dearth of information on human effects.

DR. LINDSAY: Dr. Cranmer?

DR. CRANMER: As a toxicologist trying to design experiments and interpret the results to protect the public health as well as the environment, I have a few comments with respect to the problems of the Environmental Protection Agency.

Dr. Hammer has just spoken about occupational exposure. Certainly if we had some information on the effects of occupational exposures to humans we would have information which would perhaps be of use for predicting the chronic effects of acute exposure or acute effects of acute exposure.

However, I would hasten to add that we really should increase the surveillance of the general population, because the general population and occupationally exposed are not exposed to the same compounds in the same relative amounts.

We have discussed several times here the fact that we have a biological modification of these compounds. Therefore, when you eat coho salmon or chickens you are not getting the same profile of compounds as you get when occupationally exposed to the same parent formulation. This leads to problems in setting up experiments. What are we doing wrong in toxicology?

Well, as you know, we usually utilize analytical grade materials that are going to be analyzed by gas chromatography. What we should be doing is feeding animals the material which humans would be eating.

I would also say that what we really want to do is measure the total impact, and we really need, that is the Environmental Protection Agency needs, to follow the routes through the environment and test representative organisms along the way. For example, we brought up the problem of diaposal incinerators. We are going to get different products after incineration, catalytic processing or reprocessing paper. Certainly carbonless carbon paper may originally have 4 percent of a certain formulation, but it does not come out that way when it is reprocessed.

These are things that perhaps we should consider. And if I could take one more minute of your time I think there is a great deal to be said for research into ranges of sensitivities mediated by age, sex, interactions and genetic differences.

We are dealing with 200 million people, we set up the better experiments with a few hundred animals. The sensitivities of species are variable, just as are the ages. The difference in toxicity of PCB to the mink and rat are enormous. Certainly the weanling rat is more susceptible to the induction of microsomal enzymes than older rats. Diets affect toxicity as demonstrated in the vitamin C and protein deficient animals. This certainly markedly changes the ability of these compounds and to modify other compounds' action, brings into play interactions with pesticides, drugs and alcohol. There is a considerable amount of information available suggesting that polychlorinated biphenyls alter many of these actions.

I would say when you take all the toxicology data available and take some of these variables into consideration, one concludes that we really have very little information relating to potential health effects to say nothing of the long-term environmental problems.

DR. LINDSAY: Thank you.

This reminds me of two oversights. One is I had asked Dr. Gordon to make some statements, and then overlooked him on the list that I had.

The other one was that I had tried to, on several occasions, reach Dr. Raymond Moore, who is now in the Institute of Occupational Safety and Health, to get him here today, and failing in those two or three times, I am afraid I lost track of it and I did not follow through.

So that is why we do not have him here, or someone from Occupational Safety and Health.

Dick?

DR. GORDON: I have the same kind of feeling that Dr. Nelson was developing at lunch, that today is sort of an opening warmup meeting and it

could very well be that after circulating the papers, thinking, looking at the transaction, maybe reading the newsletter, we might want to schedule a couple days more of an in-depth scientific kind of meeting in the fall. I feel that there is a lot of stuff that is in the works, and I think that some wider notification would be appropriate of some of the conclusions today to the scientific community and might be useful.

You can always say, "Well, one more symposium," but there are national meetings and there is of course an in-depth meeting that could be summoned possibly through OST or CEQ.

I just sort of feel that the review of data over time and the experiments in progress would be quite useful and I feel that that might be an appropriate thing to consider doing in three to six months.

DR. LINDSAY: Thank you.

Nick, do you want to say something?

DR. BOOTH: I don't know how important this is, Dr. Lindsay, but someone mentioned a while ago that they would be interested in human toxicity data. In the library yesterday I came across an article in one of the recent bulletins of environmental contamination and toxicology. There was a reference that went back to 1939, and it was of interest to me today to learn that there wasn't a great deal of information about toxicity of these compounds to man. And I was curious about this citation, because it said that there were three deaths that had occurred from PCBs, and I think and similar compounds, and I believe it was in the Journal of Industrial Hygiene and Toxicology. It was unavailable in our library so I didn't get to check that out.

DR. LINDSAY: Dr. Haley?

DR. HALEY: Talking about behavioral studies that the wildlife people are making, it is a very simple thing for anyone to set up what I call a pidgeon pecking routine and study all levels.

Now this is again a bird, so I would suggest that might be an appropriate type of approach to the psychological effects of these compounds. There is one thing that seems to have slipped under the floor boards. Nobody has talked about the possibility of the inhalation hazard from these compounds. We are talking about incineration, where this will be disseminated out and be in the atmosphere for people to breathe. I am not so sure they don't even get it when they are filling some of these gadgets at Monsanto, or wherever else they are doing it.

I would suggest a full scale program on the inhalation toxicology of the PCBs be undertaken.

DR. LINDSAY: EPA, take note.

Dr. Hammer, you said you had gotten some out of the particulate sampling from the air, too.

DR. HAMMER: I would like to tell my own experience the past year, and underscore another way of getting together which wouldn't involve any formal committee's business but simply just taking advantage of each other's work and ability and sometimes even reading what each other writes or calling each other on the phone, that one place where one really does have a need for a multidisciplinary approach is in the trace or toxic substances, metals, synthetic organic materials, pesticides. And I have availed myself quite liberally of the many excellent studies that have been done in terms of animal husbandry and in terms of botany, U.S. Geologic Survey, Water, FDA diet studies, to try and get some estimates of human exposure.

I think that although there is a lot more information that needs to be done, that there are already many people attacking this problem from many different ways, from many different routes. So that is the first point, that you can use this for our own studies and also for modeling.

The second point is that EPA recently published a summary of the Helena Valley area environmental pollution report and this will be published in the fall. This is one area where again due to the requests of the State of Montana, people from many disciplines went out and studied a single geographic area.

In this case there was a source, a smelter. And in addition to the gaseous pollution they studied several heavy metals, and many of us who were there were studying people and the environment animals. Whatever it is, I think we ought to consider when we plan our new studies to see if we can perhaps do our studies in the same place or in similar places, at least, and so achieve better coordination. This wouldn't always involve formal commitments.

It's an attitude, an awareness that we can and must develop. Permitting us to get together so that several years from now we will have some answers to these questions rather than getting off in different directions again.

DR. LINDSAY: Dr. Morris?

DR. MORRIS: Mucrell Morris, Food and Drug Administration.

Many of us here have suggested that we have several more years of research before we know what to do about the PCBs. One speaker discussed the proposed new legislation on toxic chemicals. May I suggest that, under our present Food Additive Amendment, either Mr. Les Ramsey

or Mr. Reo Duggan could, by tomorrow morning, draft a regulation which would eliminate the use of PCBs in food plants and feed mills, the latter being the principal known route of entry into our food chain.

Rather than quote the law, a copy of which I have before me, I will present it in simple words. The Food Additive Amendment was specifically designed and enacted to prevent unsafe levels of non-food substances from getting into foods, either by direct addition or indirectly by what is commonly referred to as migration. The Amendment specifically provides coverage of all non-food substances used in producing, preparing, processing, treating, manufacturing, packing, packaging, holding, and transporting food.

It has been reported here and elsewhere that PCBs are currently being used as heat exchange fluids in feed mills, and that this "intended use results, indirectly, in it becoming a component of food." Since it is a non-food substance which is not generally recognized as safe, it is by law a food additive. And since its safe use in feed mills is not prescribed by a food additive regulation, it is also by law an unsafe food additive, the use of which is a prohibited act because it leads to the adulteration of animal feeds. Thus, the law on food additives not only provides for the seizure of products contaminated indirectly by unsafe food additives, it also prohibits acts which might lead, indirectly, to the contamination of foods with unsafe food additives. We do not have to look only to our seizure powers in order to prevent contamination of our food supply with unsafe food additives. I do not mean to imply by this suggestion that we could have acted earlier, i.e., before we had the facts which have come to our attention as a result of this conference.

DR. LINDSAY: Reo is not here, and Les is. I know what Reo would say first, "Could you enforce it if you had it?"

Dr. Kraybill?

DR. KRAYBILL: I detect from looking at the toxicity data that there is some disparity in our findings. Some of us have discerned these differences. Again, I hate to reiterate our whole experience with the dioxins on identification and establishment of purity and I would like to have some comments from the chemists here, perhaps Mr. Cook would like to address himself to this point. For example, with 2,4,5-T it depends a lot on how you process this compound and what temperatures you are using in the processing. So what I am getting at here is that we encountered such a problem in the dioxin testing. With four different agencies in testing programs, we ran profiles or we at least identified the type of compound we were working with as standards. This might be a useful suggestion to these investigators here, if they get together and indicate what type of Aroclor they are dealing with and how much contaminant is in that Aroclor, because this will affect the end result of the toxicological response.

DR. LINDSAY: Chemist respond?

MR. COOK: There has to been an overlap of chemistry and toxicology. In the bureau we have synthesized a number of chlorodioxins, 2,3,7,8-symmetrical tetra which you will normally get if you produce 2,4,5-T from trichlorophenols. That is the tetra which is fantastically toxic. It is down in the microgram per kilogram range, whereas our most toxic pesticides generally are in the range of milligrams per kilogram. Some of the other chlorodioxins are far, far less toxic than that, by a factor of 2,000.

We do not know whether we might run into wide variation in toxicity in the group of compounds that make up the large number of polychlorinated biphenyls. You would have to separate out each member of the series and test for toxicity. There may be a situation similar to toxaphene. There are 50 to 100 different chlorinated compounds in toxaphene that are closely related chemically. In producing toxaphene, the percent of chlorination is extremely important. When chlorinated up to 50 percent, for instance, the material is not very toxic. When chlorination is in the range of 67 to 69 percent the product is a highly toxic insecticide. Over 69 percent chlorination there is a rapid fall off in toxicity.

Now back to the chlorinated biphenyls, theoretically there are 210 possibilities for different isomers. There are people who are separating these and my hope is that somebody will do some individual isomer toxicity testing, which I think would be very important. I think this would add a good deal to our information. All these different isomers are not present in the different Aroclor products. Arochlor 1242 will have a number of compounds in common with 1254. I don't think 1221 has much in common with 1268. But there are overlaps. And if one were to pick out the important individual compound in the Aroclor for which we do have some toxicity data, we would be a step ahead.

DR. LINDSAY: Thank you.

DR. LINDSAY: Dr. Riley?

DR. RILEY: Dr. Riley, CSRS, Department of Agriculture.

I haven't heard the question brought up today, and perhaps it ought to be considered, what are the alternatives to the PCBs.

Now, it came out during our discussion of these heat exchange fluids that there are more expensive materials that can be used in processing plants. Do we have good information in this area as to alternate materials, substitutes for PCBs and what their cost would be? What are the long-term projection for when alternate materials can be brought into use?

The indications that we seem to be getting in this conference, and what we are hearing from people who are working with these materials are that this is an extremely persistent chemical; and it is going to be in the environment for a considerable period of time.

Thank you.

DR. LINDSAY: Thank you.

Dr. Plimmer?

DR. PLIMMER: Dr. Plimmer, ARS, U.S. Department of Agriculture.

I would like to draw your attention to one point in the Monsanto technical bulletin on aroclors. This follows up Dr. Cooks' comment. For nearly every Aroclor mentioned, a darker, less pure variety exists with about the same physical and chemical characteristics, but lower in price. So I think that somehow, our toxicological study should be related to these two grades.

We talked to Monsanto about the dangers of oxygenation in the higher grade aroclors to give dibenzofurans, but we don't know anything about the lower grade aroclors, what their degree of purity is and whether they could contain dibenzofurans.

This is one point.

The second point I would like to emphasize is that for these investigations, and I don't know whether their synthesis is currently being

attempted, but we need some radioactively labeled aroclors if we are going to follow the environmental fate of these compounds.

DR. LINDSAY: Thank you, Dr. Plimmer.

Anyone else?

Dr. Hoopingarner.

DR. HOOPINGARNER: This is a comment I would like to direct, maybe at the Patuxent group. They said that they have seen polychlorinated biphenyls since they have been analyzing tissues. I commented about the coho that had died the first year, apparently from DDT poisoning, at least it gave those symptoms. Since that time, they give none of the DDT symptoms. Yet the level of DDT in the coho has not changed.

DR. LINDSAY: Dr. Stickel?

DR. STICKEL: I don't believe that anybody who has not been close to the coho salmon probably is going to be very wise to say even one word about it.

DR. LINDSAY: Are there other questions or comments? Dr. Flick?

DR. FLICK: I would like to just mention once more, and that is again drawing on the dioxin problem of the past, perhaps still current, is the setting up of a long term study with a view to determine if after exposure, is there actual recovery. Now we had -- we did this with the dioxines in a short term study and we found that after removal of the animals from any dioxins, that their recovery did not occur. In fact, the disease even became more pronounced and more grossly manifested. So I think recovery --

DR. LINDSAY: You mean of the ones that have been continuously exposed?

DR. FLICK: Yes -- and they were compared with controls that were continued on dioxins. So this type of approach perhaps could also be carried over to PCBs to find out if indeed the compounds, radio tagged or not, persist in tissues, and are manifesting toxic symptoms some time after removal.

DR. LINDSAY: That is tough. You are damned if you do and if you don't.

Dr. Harris?

DR. HARRIS: I would like to say a few words.

Dr. Gordon proposed one question, is there a clear and present danger? I don't believe that has been resolved. We are going back and doing a lot more pharmacology and toxicology on this, and it might be another year before we gain more knowledge.

We had lethal effects on embryos, although they were chicken embryos, at one part per million, and although the hen's food received 20 parts per million, to me that is a hazard.

And the people that processed this fishmeal are not schooled in precautions of using it; they are completely ignorant of the hazardous material they are dealing with. They knew of the leakage, and Monsanto knew it; that is why they continued to ship more and more in, without any precautions.

So I believe we have a present and clear danger. Time won't wait until you can generate a year's toxicology.

DR. LINDSAY: True.

DR. Kimbrough?

DR. KIMBROUGH: I wonder if there would be any point in doing some liver function studies and some other studies in the workers that have been working in the fishmeal processing plant, and whether this could be done maybe by the plant or by the State Health Department, and of course, they should also be checked for chloracine. Other symptoms of toxicity include alcohol intolerance complaints of the GI tract, nausea.

DR. LINDSAY: That is always tricky, of course, going into a situation like that and getting the compliance of the company to build up a nice suit against themselves later on.

DR. HARRIS: This is owned by the Peruvians. They have a different ballgame.

DR. LINDSAY: They would probably load up some of our shrimp boats down there.

Are there any other questions?

A little while ago Dr. Burger was very generous with his comments to me personally, and I am very much afraid mistakenly, becuase I don't deserve any compliments here; you do - the people who came. We set this up in a big hurry, and everyone who has contributed so generously of his time and thoughts to the conference deserves the compliments that Dr. Burger passed on to me, and I'm sure he doesn't mind me giving them to you on his behalf.

I certainly appreciate you having come today. I hope that more will come from this meeting, that individual sessions tailored to the particular talents and problems will emerge, and that from this we'll all be better off.

Thank you very much for coming.

(Whereupon, at 3:30 p.m., August 5, 1971, the conference was concluded.)

APPENDIX I

Polychlorinated Biphenyls:

Distribution and Storage in Body Fluids and Tissues of Sherman Rats

MONS 069255

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MONS 069256

Abstract

An analytical method for determination of PCB (Aroclor 1254(R)) in blood, urine, feces, and tissues of Sherman strain rats after prolonged dietary intake (100, 500, and 1000 ppm) is presented. The method involves homogenization and extraction with hexane, cleanup on a silica gel microcolumn and analysis by electron-capture gas-liquid chromatography. Recovery data are shown at 0.1, 5, 14, and 100 ppm. Mass spectroscopic characteristics of PCB in tissues are given. Distribution, storage, and excretion rates are presented during feeding and after discontinuance of PCB in the diet.

In 1881, polychlorinated biphenyls (PCB's) were first described by Schmidt and Shultz.[1] Commercial production was begun in 1930.[2] These compounds have been and still are widely used as plasticizers and resins and in chlorinated rubber.[3,4] They are known to enhance the insecticidal properties of lindane[5], DDT, and dieldrin.[6] They may be used as stationary phases in gas-liquid chromatography.[7]

Their widespread use may have resulted in contamination of the ecosystem as suggested by many recent reports. Aroclor and DDT were both used during World War II. In 1948, total organic chlorine analysis provided data for the first determination of DDT storage in man.[8] Since this method has no specificity for DDT, the total organic chlorine content would not preclude the presence of other organic chlorine-containing moieties. In terms of halide specificity, the Dohrmann microcoulometric gas chromatographic method for detecting pesticides was well established by 1960[9], and electron-capture was in use by 1961.[10] Roburn[11] first observed extraneous peaks in 1965; he used a potentiometric mode of analysis for total chlorine and found disagreement between this method and total chlorine content calculated from gas chromatographic results in which Argon ionization and electron-capture were used for the analysis of tissues and eggs of wild birds. These samples showed significant amounts of unknown electron-capturing compounds. Roburn postulated that these were metabolic intermediates of one or more of the organochlorine pesticides. These differences could have been indicative of PCB contamination. It was not until 1966 that S. Jensen[12] using electron-capture and mass spectrometry was able to identify unknown peaks found in fish and a bird carcass as PCB's. Undoubtedly, the time lag between use and detection of PCB in the environment is a matter of accumulative concentration and/or instrumentation development.

The presence of PCB's in environmental samples has been well established.[13, 14] Acute and subacute feeding studies to determine toxicities and some residue levels have been done with rats, birds, and guinea pigs.[15,16,17,18,22] Tolerance studies have been done with fish and other forms of marine life.[19, 21,22] Pathological changes in the liver and other organs have been shown in guinea pigs, rats, and rabbits at high concentrations.[22] Hydropericardial edema and growth depression proportional to dietary levels of the toxicant have been produced in the chicken.[23] PCB's are known to affect estrogen levels in birds which in turn reduces calcium reserves and results in thin eggshells. Chemical porphyria from oral administration has also been shown in chicks.[18]

This paper presents distribution, storage, and excretion rates of Aroclor in certain tissues, body fluids, and excreta of rats following 1) a single oral dose, 2) repeated dietary intake, and 3) after discontinuance of PCB in the diet.

Methods

Aroclor 1254(R) and 1260(R) were supplied by the Monsanto Chemical Company with respective lot numbers, AK-38 and AK-3. Dietary formulations were prepared according to the methods described previously by Gaines and Kimbrough.[24]

Experiment A: Aroclor 1254(R) and Aroclor 1260(R) were administered at 1600 mg/kg and 3200 mg/kg, respectively as a single oral dose, by stomach tube to three female, 173- to 183-day-old, white Sherman rats weighing 260-318 g. Four control rats, two in each group, were given peanut oil only. Twenty-four hours later the animals were sacrificed, and blood (oxalated), fat, muscle (abdominal), liver, kidney, lung, and brain were taken for analysis.

MONS 069259

Experiment B: Aroclor 1254(R) at 1000 ppm was fed for 98 days to 29- to 34-day-old Sherman strain male and female weanling rats, in groups

of 10. Controls were fed only plain chow. Food consumption was measured at intervals as described previously by Kimbrough and Gaines.[42] All rats were weighed on a weekly basis from experiment initiation until time of sacrifice or death. The rats consumed an average of 72.7 mg/kg/Aroclor per day.. Blood (oxalated), fat, muscle (abdominal), liver, kidney, lung, and brain were taken from each rat at time of sacrifice. Tissues were preserved in 10% formalin and held for analysis.

Experiment C: Sherman strain weanling rats (61-to 66 days-old) were fed a diet of plain chow or chow fortified with Aroclor 1254(R) at a concentration of 100 ppm for 58 days. Fortified chow was discontinued, and samples were collected during the recovery period. Food consumption was measured at 2-day intervals for a period of 8 days; after 42 days food consumption was measured daily for 4 days. Surviving rats were weighed once a week. At various times during the experiment rats were individually housed in metabolism cages. Urine and feces were collected over a 16-hour period during which time plain chow was fed. Following excreta collections, rats were sacrificed. Blood (oxalated), fat, muscle (abdominal), liver, kidney, and brain were taken from each rat at time of sacrifice. Tissues, urine, and feces were frozen at time of collection. Whole blood was centrifuged and the plasma refrigerated. The outline of the experiment as aforementioned is summarized as follows:

These animals (two on the diet and one control) were sacrificed on day 4, 9, 13, 17, 25, 34, 42, and 58. After discontinuance of PCB in the diet, two animals were sacrificed on day 8, 16, 24, and 71.

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Experiments D & E: Sherman strain weanlings 36- to 41 days-old) were fed Aroclor 1254(R) at dietary levels of 100 ppm or 500 ppm for 252 days. Body weights were determined initially and weekly thereafter for 121 days. On the basis of food consumption measurements, the average rates for intake of Aroclor 1254(R) were 6.8 mg/kg/day and 36.4 mg/kg/day for 100-ppm and 500-ppm dietary levels, respectively. Samples were collected in the same manner as Experiment C.

Sample Preparation

Polychlorinated biphenyls like DDT, are not water soluble but are, in fact, highly lipid soluble. Solvents for tissues and plasma extractions were selected for this property.

Plasma (at least 2 mls) was extracted by the method of Dale et al.(25) Later in the study, the Bryant-Thompson modification of this method was used.(26) No significant difference has been found between these methods in this laboratory with the exception that convenience favors the Bryant-Thompson modification.

Samples of fat (250 mg), liver, muscle, kidney, and lungs (500 mg each) were homogenized two times in 4 ml of hexane, and the extracts were combined.

Brain (500 mg) was homogenized two times in 4 ml of acetone, and the extracts were combined.(27)

Urine (mean volume of 10 ml) at pH ≤ 7 (using pHydrion paper) was diluted to a volume of 25 ml by the addition of water and 1 ml isopropanol. These samples were extracted in 125-ml separatory funnels three times with 10 ml hexane. The hexane extracts were combined, washed once with 3 ml of water, and dried with a minimum amount of anhydrous sodium sulfate.

Feces were desiccated over calcium chloride for 1 week. One gram was

then pulverized in a mortar and pestle and extracted for 1 hour with 25 ml of acetone on an automatic shaker. The acetone extract was decanted and placed in a 50-ml centrifuge tube. Particulate matter in the extract was removed by centrifuging and/or decanting. The extracts were evaporated with a stream of dry nitrogen to a volume of 0.5 ml in a water bath (40°C). The acetone extracts were evaporated just to dryness and made to a volume of 0.5 ml in hexane. The samples were dried with anhydrous sodium sulfate and held for cleanup and analysis by electron capture gas-liquid chromatography.

Preparation of Silica Gel and Micro-Column

Silica gel[27] from Woelm (Activity grade 1) was activated at 130°C for 4 hours and desiccated over calcium chloride for 16 hours before deactivation at 3% (V/W) by the addition of water. The silica gel was allowed to equilibrate at least 2 hours before use. After comparing the weight of the silica gel before and after activation, it was found to be activity grade I as specified by the manufacturer and further activation was unnecessary; however, each new batch was routinely activated.

One gram of the deactivated silica gel was placed in a disposable pipet, which had been loosely plugged with silanized glass wool, and gently seated. Columns were pre-washed with 10 ml of hexane. Each sample was placed on the column in a volume of 0.5 ml. At least 1 ml of hexane was used to quantitatively transfer the sample to the head of the column. The column was eluted with 10 ml of a 1:1 benzene:hexane mixture.[27] Samples were chromatographed in sets of eight with duplicate column blanks. Aroclor 1254(R) standards were eluted simultaneously in order to ascertain the variance in silica gel activity and to detect any possible contamination from solvents, glassware, or silica gel. Samples from experiments A, C, D, and E were treated as above

and analyzed by electron-capture gas-liquid chromatography under one of the following conditions using a MicroTek MT-220 gas chromatograph: pulse mode (using dual columns and dual detectors); cell voltage, 36.5 volts; pulse width, 5.5 μsec; pulse rate, 120 μsec; detector, H^3, parallel plate design, No. 1 205°C, No. 2 198°C; column, 6' x 1/8" O.D. U-shaped pyrex glass packed with 5% OV 210 on 80/100 mesh Chromosorb W, high performance, 170°C; inlet 238°C; carrier gas, argon-methane (95-5%), 50 psi; flow-through Detector No. 1, 50 cc/min; Detector No. 2, 60 cc/min. The DC mode was as follows: cell voltage, 15 volts; detector, H^3, parallel plate design, 210°C; Column, 6' x 1/4" O.D. U-shaped pyrex glass packed with 1.5% OV-17/1.95% QF-1 on 100/200 mesh Chromosorb W, HP, 200°C; inlet, 230°C; carrier gas, nitrogen, 40 psi; flow-through detector, 75 cc/min.

Samples from Experiment B were injected directly as hexane extracts and analyzed by electron-capture gas-liquid chromatography under the following conditions using a MicroTek MT 220 gas chromatograph: cell voltage, 40 volts DC; detector, Ni^{63}, 250°C; column, 6' x 1/8" O.D. U-shaped glass column packed with 1.5% OV-17/1.95% QF-1 on 100/200 mesh Chromosorb W, HP 200°C; inlet, 210°C; carrier gas, nitrogen, 40 psi; flow-through detector, 80 cc/min, 20 cc/min scavenger plus 60 cc/min carrier gas flow.

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Evaluation of Methodology

The efficiency of the silica gel micro-column and the extraction techniques employed were evaluated by determining the recovery of Aroclor 1254(R) from in vitro fortification.

Recovery studies were completed using the following methods for control tissues, plasma, and 48-hour urine and feces collections. The fortification level for each type of sample was the mean concentration, with the exception of fat, of Aroclor 1254(R) found for that sample in Experiment C; fat samples were fortified with 100 ppm.

Tissues (fat, liver, kidney, brain, and muscle): Aroclor was added directly to the hexane extract prior to liquid chromatography.

Plasma: Aroclor was added directly to fresh plasma, thoroughly mixed using a mini-mixer, and allowed to equilibrate overnight under refrigeration. Samples were then treated as mentioned in methods.

Feces: Aroclor was added directly to pulverized dry feces and treated as mentioned in methods.

Urine (three slightly different approaches were used):

No. 1- Aroclor was added to 1 ml of isopropanol, then mixed with a solution of urine and water (10 ml + 15 ml) and analyzed as mentioned above; No 2- Aroclor was added to a hexane extract of 10 ml of urine which had been prepared as mentioned above; No. 3- Urine (5 ml) was added to a known quantity of Aroclor in a 15-ml centrifuge tube, mixed on a minimixer, and allowed to equilibrate at room temperature for 16 hours. Urine was extracted three times with 3 ml of hexane; the hexane extracts were combined and evaporated to 0.5 ml.

All samples were eluted from a silica gel micro-column. Recovery data for all samples are presented in Table 1. All in vitro recoveries, whether from liquid chromatography or a combination of extraction and liquid chromatography, are at an acceptable level with the exception of urine. Recoveries of DDT from human urine using liquid-liquid extraction techniques have been reported by Cueto & Biros[28] and Cranmer et al.[29] with recoveries of 72 and 96%, respectively. In both instances, their extracting solvent or combination of solvents was more polar than hexane. The recovery in approach No. 1 and possibly 3 might be attributed to the partition coefficient of hexane in an aqueous system. In order to evaluate this, reciprocal p-values were determined using

the techniques of Bowman and Beroza.[30] Aroclor 1254(R) (1.787 µg) was then added to 5 ml of a 25:1 water:isopropanol mixture which had previously been allowed to equilibrate with hexane overnight. (A mixture of water + urine: alcohol in the same ratio, was used in Experiment C.) Five ml of hexane was added to this solution and the two immiscible phases mixed for 1 min using a mini-mixer. The layers were allowed to separate, and an aliquot of the hexane layer was analyzed by electron-capture. The average of triplicate analyses gave a reciprocal p-value of 0.764, or 76.4% of the Aroclor was partitioned into the hexane layer from the aqueous system. This value agrees favorably with approach No. 3 but does not account for the 17% difference in approach No. 1. Zitko[20] states that he found fractionation taking place when emulsions resulting from mixing Aroclor 1254(R) with water were broken by centrifugation. Gas chromatographic analysis of a hexane extract of the supernatant showed it to be richer in the lower chlorinated biphenyls than the original preparation. This varied with batches of Aroclor. When a peak-for-peak comparison was made, whether in vitro fortification or p-value studies were being considered, recovery was generally low for all constituents, and the average recovery was reported. Low recovery from the urine was probably due to the extracting solvent. The values reported were not corrected for in vitro recoveries.

Interference of DDT-PCB

The presence of PCB's, DDT, and DDT-like moieties in samples presents quite a problem both qualitatively and quantitatively during analysis. Initially, samples of liver and fat from control rats for each sampling period were analyzed for DDT and its metabolites; samples were taken from the oldest control rat in the study. These tissues usually showed measurable amounts of DDT, DDD, and DDE. The values for liver and fat, respectively, are as follows: p,p'-DDT, 0.046 and 1.366 ppm; p,p'-DDD, 0.036 and 0.546 ppm; p,p'-DDE, 0.016 and 1.098 ppm.

Since preliminary evaluations showed liver and fat storage of PCB's to be a minimum of 10 to 100 times the above mentioned values, interference was considered to be nonexistent. However, three possible approaches to separation of PCB and DDT-like materials without any chemical degradation of the pesticides concerned were evaluated. Thin-layer chromatography was considered first. Using silica gel G and essentially the techniques as outlined by Walker and Beroza[31], both Aroclor 1254(R) and Aroclor 1260(R) in the presence of the chlorinated hydrocarbons, p,p'-DDE, p,p'-DDT, o,p'-DDE, o,p'-DDT, dieldrin, and heptachloride epoxide, moved with the solvent front. Using polar and non-polar mobile phases, the Rf values for PCB's and p,p'-DDE were identical and not resolved. This can possibly be used in confirmation and identification of Aroclors.

The two other approaches utilize column chromatography. Florisil was used, as proposed by Reynolds[32], in which PCB's are eluted from the column with hexane, while other chlorinated hydrocarbons are eluted with a diethyl-ether:hexane mixture. Reynolds evaluated Florisil with a mixed standard of Aroclor 1254 and a chlorinated hydrocarbon mixture containing lindane, heptachlor, aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, p,p'-DDD, and p,p'-DDT. Aldrin, p,p'-DDE, and heptachlor are eluted in the PCB fraction. Although these pesticides were not separated, this approach might still be acceptable since aldrin and heptachlor are seldom found in environmental samples in their unepoxydated forms. However, when an attempt was made to reproduce the work of Reynolds using PR grade Florisil, a mixture of Aroclor 1254(R) plus the chlorinated hydrocarbons, lindane, heptachlor epoxide, dieldrin, p,p'-DDD, p,p'-DDE and p,p'-DDT, measurable quantities of p,p'-DDT, p,p'-DDD and lindane were found in Fraction 1, i.e., 66, 10, and 32% of the total, respectively.

Reynolds does not mention the quality of the Florisil although he does mention how it was handled. The problem, however, seems not to be related to the grade of Florisil. Bevenue and Ogata[33] have presented data using both qualities of Florisil and were unable to reproduce the work of Reynolds. There is some disagreement between the fractioning pattern of Bevenue and Ogata[33] and the one observed in this work. This could probably be due to the quality and treatment of the Florisil. These problems suggest that the elution characteristics of Florisil should be evaluated before use for separating PCB and DDT.

Armour and Burke[34] used a column of silicic acid (treated) and Celite 545. The column is eluted first with petroleum ether for PCB's and then with a mixed solvent system of dichloromethane-acetonitrile-hexane (80:1:19) for the chlorinated hydrocarbons. This system has been evaluated using standards at levels below those that would be expected in environmental samples, i.e., 0.5 ppm Aroclor 1254(R) and Aroclor 1260(R) and 0.1 ppm γ - and β-isomer of BHC, heptachlor epoxide, o,p'-DDE, p,p'-DDE, dieldrin, p,p'-DDD, and p,p'-DDT. Separation has been a problem with the p,p'-DDE; Fraction 1 contained 20% p,p'-DDE with the remaining 80% in Fraction 2. Although some recoveries were less than 85%, all other pesticides evaluated were only found in Fraction 2. According to Armour & Burke[34] the elution pattern can be altered by the water content of the silicic acid. P,p'-DDE was the first affected in the group of pesticides that were used to show these changes.

In Vivo Recovery Study

An attempt was made to account for the total dosage of Aroclor using individual rats.

(1) Aroclor intake was calculated from average food consumption and diet fortification level.

(2) Fat tissue was assumed to constitute 10% of the average body weight of each rat.

(3) Whole blood volume was taken as 6.7 ml/100 g of body weight, and the hematocrit was then used to calculate total plasma volume.

(4) Total weight of each sample collection of feces and urine for a 16-hour period was used.

A maximum of 30% of the Aroclor intake was recovered under these conditions. As time on the diet increased, recoverability decreased; this could indicate that a great deal of Aroclor was being stored in tissues other than those analyzed and/or that the excretion of Aroclor would have been greater with time.

Reagents and Apparatus

Silica gel	M. Woelm Activity Grade I treated as mentioned in methods. (Waters Associated, inc. Framingham, Mass. 01701).
Sodium sulfate	Baker and Adamson Reagent grade treated according to H.F. Enos. Perrine Primate Research Center, Perrine, Fla.
Water	De-ionized and double distilled. Extracted three times with n-hexane before use.
Hexane	Nanograde quality, no treatment.
Benzene	Nanograde quality - stored over Na^{0}.
Glasswool	Silanized glasswool from Analabs (North Haven, Conn.)
Micro-columns	Disposable capillary pipets, 5 3/4" length (Owens-Illinois, Toledo, Ohio).
Receivers	Glass stoppered 15 ml centrifuge tubes.
Homogenizer tubes	Thomas Scientific Apparatus (Phila. Pa.)
Tri-R Stirrer	Eberbach Corp. (Ann Arbor, Mich.)

Results and Discussion

Routinely, this laboratory operates dual channel solid state electrometers at a sensitivity of 4×10^{-10} amps full scale which permits the detection of 10 to 30 picograms of the more common chlorinated hydrocarbons. There was a significant difference between the response characteristics of the Aroclors as compared to the chlorinated hydrocarbons. To achieve a representative gas chromatographic trace, at least 350 and 490 picograms of Aroclor 1254(R) and Aroclor 1260(R) were required. A gas chromatogram illustrating the difference between the electron-capture response characteristics of these compounds as compared to chlorinated hydrocarbons is shown in Fig. 1.

Experiment A

Data resulting from electron-capture analysis of plasma and tissues in Experiment A are presented in Table 2. Quantitation is an approximation. In all cases, the peak heights (in mm) of all PCB components were summed for the respective standard at a given picogram quantity injected. A response factor was determined for each standard in picogram/mm. For each sample, a summation was made of the total response (in mm), and the product of this times the response factor calculated to give picograms of PCB or PCB-derived material in the sample. When the gas chromatograms for the samples were compared with those of the Aroclor standard, there were no recurrences of the general Aroclor pattern. Some peaks had completely disappeared. This was also true for each of the succeeding studies.

Although the dosage level of Aroclor 1260(R) was twice that of Aroclor 1254(R), the mean storage levels after 24 hours were essentially the same. Considerable variation in the storage levels was found in each group. Differing absorption rates from the gut might account for the variation in levels

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found; variation was highest in the fat and lowest in the plasma. According to Hayes[35], acute dosages, such as the one given here, do not result in storage, but rather in a flooding of the organism.

Experiment B

Only surviving rats were analyzed in Experiment B. Five of the ten male rats died-one on the last day (day 98), and one each on days 85 and 56 and two on day 50. Eight of the ten female rats died-one each on days 89, 80, 47, 41, and 35, and three on day 55. Tucker and Crabtree[15] fed male albino rats 1000 ppm Aroclor 1254(R) and reported similar results for 0 of 6 rats including a 21% reduction in food consumption. All survivors in Experiment B appeared to be normal; however no increase in weight was observed in surviving males after 84 days of dietary intake and after 77 days for surviving females. Food consumption for dosed males and females was 66 and 64%, respectively, of consumption by controls,

Data from electron-capture analysis of tissues and plasma are presented in Table 3. The variation in the concentrations of PCB in this experiment, as in Experiment A, is least in the serum and brain and highest in the muscle and fat. No significant difference ($p>0.50$) has been found for storage of Aroclor 1254(R) by females as compared to males. Hayes[35] and Dale et al.[36] report that storage of DDT in female rats exceeds that of male rats when both are maintained on the same diet and that the enhancement in storage exceeds the greater food intake by females.

The absence of certain peaks in the Aroclor standard when compared to PCB-derived material in the samples became more dramatic in this as compared to Experiment A. Generally, the percentage of matching Aroclor peaks found in each tissue was as follows: plasma 81%, fat 74%,

brain 66%, liver 64%, lung 63%, muscle 62%, and kidney 58%. These differences could be attributed to:

(1) selectivity in storage for each tissue

(2) enzyme activity resulting in some form of metabolism

(3) excretion favoring some isomeric forms as opposed to others.

Experiment C

The data from the analysis of tissue, plasma, and excrement obtained according to the schedule as outlined in Experiment C are graphically illustrated in Fig. 2 and 3. Apparently, there is a steady buildup of Aroclor in all tissues, while the excretion trend is quite erratic. The excretion trend may be due in part to the discontinuous collections; therefore, these results reflect only the excretion pattern at specific times. The residue levels observed in Experiment B created some doubt that levels observed after the 58-day dietary intake were representative of steady state values for Aroclor 1254(R). Hayes[35] states that DDT fed to rats at a constant rate is increasingly stored in their fat until it reaches a plateau, and on a diet containing 200 ppm or less this plateau is reached within 90 to 140 days. Dale et al.[36] found mean concentrations of DDT and DDE in the fat of male rats fed 200 ppm in the diet for 90 days to be 525 and 51 ppm, respectively, while male rats on the same diet for 140 days had mean concentrations of 506 and 42 ppm. A maximum concentration in liver, kidney, and brain is achieved within a few days for a dietary level of 1000 ppm.[35]

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Experiment D and E

Results of Experiment D showed that rats on the same dietary levels

stored more Aroclor in their tissues after 240 days than at the end of 58 days. The 240-day group showed no significant difference in the quantity of the PCB-derived material found in the urine but showed significantly more PCB excreted in the feces.

Analysis of tissue, plasma, and excreta from Experiment E tends to indicate that Aroclor storage, like DDT, is directly related to the daily dosage. However, the points at which equilibrium storage is achieved are yet to be determined.

At dietary levels of 100, 400, and 800 ppm for 2 years[37], the mean values of DDT stored in the fat of rats at equilibrium were reported as 35,1028, and 4200 ppm, respectively. The concentrations of Aroclor 1254(R) found in the fat after 240 days at dietary levels of 100 and 500 ppm were 1101 and 10,021, respectively. Generally, the levels of DDT stored in the fat of male rats after 180 days are equivalent to levels after 2 years[37], although there is some reduction in DDT storage beyond 2 years. These levels in the fat illustrate the vast differences in storage at the steady states for these two compounds, and the vast differences in their relative toxicities and/or lipid solubilities. (The brain concentration, rather than fat concentration is a better indication of toxicity),[38]

DDT storage can be reduced if exposure is reduced or discontinued. Lang and Fitzhugh[37] found retention of 50-75% and 25% of DDT in the fat, 30 and 90 days after discontinuing diets containing 5 to 50 ppm. After 58 days on a dietary level of 100 ppm Aroclor and a recovery time

of 71 days, 80% of the concentration in the fat remained. Elimination seems to parallel that of DDT.

These data indicated the concentration of Aroclor found at time of sacrifice to be in the following order: fat > liver ≥ feces > kidney > brain > plasma ≥ urine. The order of storage for tissues and plasma seems to parallel lipid content. Fecal excretion exceeds urine excretion with Aroclors as it does with DDT and other chlorinated hydrocarbons.

Although p,p'-DDT and p,p'-DDE were determined at the 0.01 ppm sensitivity level in liver from a control rat, the degree to which silica gel removes interfering moieties from tissue or excreta extracts cannot be determined at this time. Before and after elution from silica gel there usually was a marked difference in the appearance of the extracts depending on the weights of tissue and excreta used. (Law and Goerlitz [39] report the success of pigment removed with silica gel from water samples.)

Muscle and plasma were the least affected by liquid-solid chromatography and could have been analyzed very easily without this treatment. In fact, for the concentrations of Aroclor found, most of the samples could have been analyzed without any cleanup, taking into consideration the problems associated with column and detector contamination.

After elution patterns of Aroclor 1254(R) were established, 10 ml of benzene:hexane were not required for quantitative elution of PCB, in fact, only 4 ml were required. This saved time in subsequent analyses.

The elution pattern of Aroclor 1254(R) was unchanged at the fortification levels mentioned in Table 1. The Aroclor 1254(R) standard that was chromatographed simultaneously with each batch of samples analyzed had a mean recovery of 104% with a Standard Error of ± 3.1 for 23 samples.

The fact that there was not a complete peak for peak match between stored and excreted PCB and the dietary standard is not new (Fig. 4). Whether this is evidence of metabolism is yet to be determined. These differences were successfully demonstrated by electron capture gas-liquid chromatography and gas chromatography-mass spectrometry in that there were changes in the relative abundances of some of the components of Aroclor 1254(R) in fat and the urine samples. This has not yet been demonstrated in any other tissue.

The mass spectra showed molecular ions at m/e 288, 323, 358, and 392 containing Cl_4, Cl_5, Cl_6, and Cl_7 isotopic clusters in the fat and 254, 288 324, and 358 in the urine. Two additional ions which were observed in the urine only, were m/e 304 and 405. These ions contained the Cl_4 isotopic cluster.

The presence of unaltered Aroclor in the feces may be indicative of lack of absorption; this was the only sample that showed no alteration, whatsoever, when compared to standard Aroclor 1254(R)

The neutral urinary excretion pattern was quite the contrary to feces. This may in part be due to a Solubility phenomena which Zitko(20) encountered when working with PCB and water. The situation of Zitko with either urine or alcohol-water mixtures under in vitro conditions was not reproduced. Under in vivo conditions, there very well may be selective solvation of certain constituents of Aroclor. Selected samples of urine were analyzed

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by gas chromatography using the electrolytic conductivity detector in the reductive mode. The presence of chloride ion was further substantiated. The retention times of these components compared favorably with peaks in the Aroclor standard.

The effects of cellular concentrations of PCB may be as indeterminate as effects of DDT. In contrast to DDT a pure compound was not used. Isolation of the Aroclor constituents in tissues and body fluids in pure form will be undertaken to determine which of these are responsible for the many effects resulting from chronic or acute exposure to Aroclor. These data will be reported separately.

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Conclusions

It is hoped that these data will elucidate some of the problems inherent in studying the polychlorinated biphenyls: and that some correlations can be found between the residue levels reported and the LD_{50} determinations including the pathological findings to be reported in a later publication by Kimbrough et al.[40] There are some basic generalizations that can be drawn from the data presented:

(1) Following dosage with Aroclor 1254(R) or 1260(R), whether acute or chronic, residue amounts can be detected in all body tissues, fluids, and excrement.

(2) At the same dosage level rats store more PCB's than DDT.

(3) No significant difference is apparent in the storage of PCB's by male or female rats when fed the same dietary levels.

(4) PCB's are stored primarily in adipose tissue.

(5) Since there was no recurrence of the general Aroclor pattern in the gas chromatograms or total ion current traces of the mass spectrometer between the Aroclor standards and components observed in the fat and urine, possible metabolism or differential absorption is suggested.

(6) As the chlorine content in the PCB molecule decreases, toxicity apparently increases; this suggests that some components in the mixture are less stable and more reactive, thus possibly producing some metabolite.

Footnotes

The Monsanto Company manufactures PCB's chiefly in the United States under the trade name of Aroclor. They are manufactured in France by Prodelec, and in Germany by Bayer, with trade names, Phenochlor and Colphen, respectively.

These compounds are designated by numbers. The first two digits represent the molecular type: 12, chlorinated biphenyls; 25 and 44, blends of chlorinated biphenyls and chlorinated terphenyls (75% biphenyl and 60% biphenyl, respectively); 54, chlorinated terphenyls. The last two digits give the weight percent of chlorine. Thus, Aroclor 1254(R) and 1260(R), with which this paper is concerned, are chlorinated biphenyls containing 54% and 60% chlorine, respectively.

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TABLE 1

Recovery of *In Vitro* Fortification of Control Samples with Aroclor 1254

Tissue	Weight	Fortified (ppm) AR 1254	Percent Recovery *
Plasma	2.0	0.5	98.6
Fat	.250	100	82.2
Liver	.500	6.0	94.8
Muscle	.500	4.0	103.1
Brain	.500	3.0	100.4
Kidney	.500	2.0	100.6
Feces	1.000	14.0	99.8
Urine (Approach 1,2,3)		0.1	62.0
		0.1	114.0
		0.1	77.0

* Plasma, fat, liver, miscle, brain, kidney, feces - mean of 4 determinations, and urine $\bar{X}$ mean of duplicate determinations.

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TABLE 2

Storage of Aroclors 24-hours After Oral Ingestion by Stomach Tube

	Aroclor 1254(R) (dosage level - 1600 mg/kg)	Aroclor 1260(R) (dosage level - 3200 mg/kg)
Plasma		
Mean	24.03	15.79
SE	± 8.08	± 0.99
Brain		
Mean	138.00	145.17
SE	± 36.41	± 23.50
Fat		
Mean	1146.9	930.0
SE	± 574.5	± 426.6
Liver		
Mean	141.20	236.1
SE	± 47.80	± 116.3
Kidney		
Mean	274.03	328.5
SE	± 30.47	± 114.9
Lung		
Mean	65.91	105.17
SE	± 29.48	± 21.13
Muscle		
Mean	80.29	37.00
SE	± 67.58	± 22.37

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TABLE 3

Distribution of PCB-Derived Material Following 98-Day Exposure to a Dietary Level of 1000 PPM Aroclor 1254(R)

	Males (ppm)	Females (ppm)	Males vs. Females
Plasma			
Mean	17	18	$p > 0.50$
SE ±	6	5	
Fat			
Mean	11278	8431	$p > 0.50$
SE ±	5742	579	
Muscle			
Mean	155	753	$p > 0.20$
SE ±	117	721	
Lung			
Mean	78	52	$p > 0.50$
SE ±	23	20	
Brain			
Mean	111	94	$p > 0.50$
SE ±	14	21	
Kidney			
Mean	56	54	$p > 0.50$
SE ±	9	24	
Liver			
Mean	155	210	$p > 0.20$
SE ±	30	13	

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Fig.1

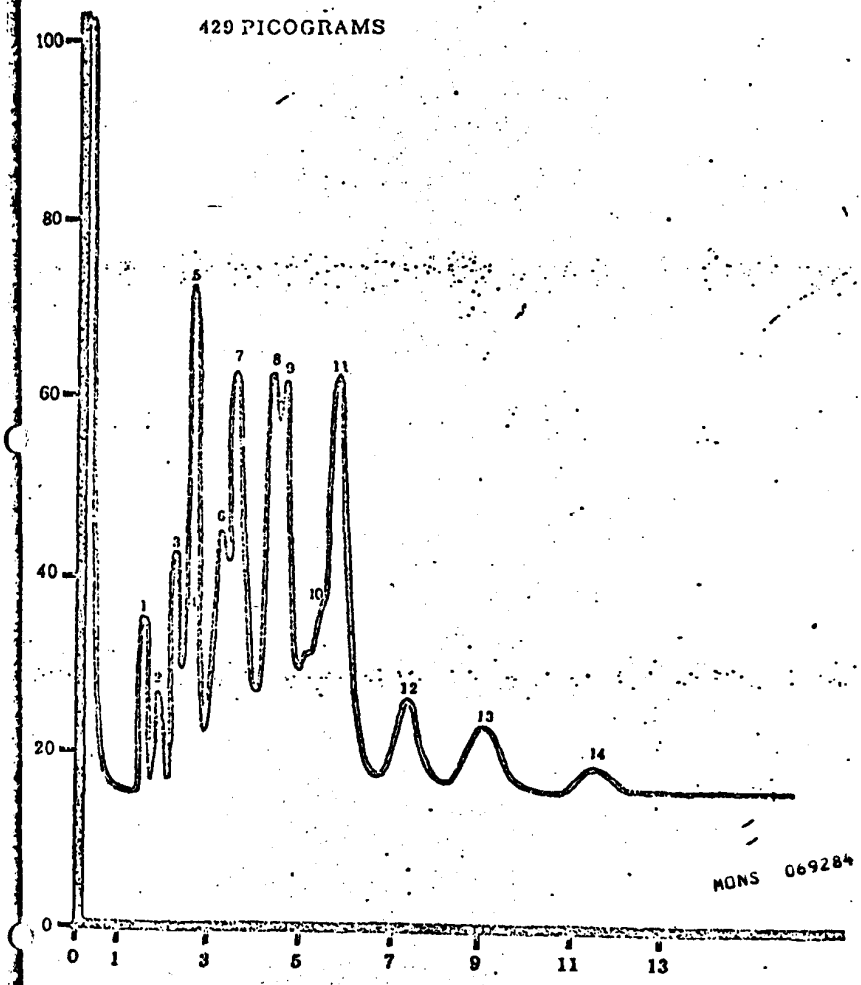
AROCLOR 1254
429 PICOGRAMS
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
1
3
5
7
9
11
13
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Fig. 1

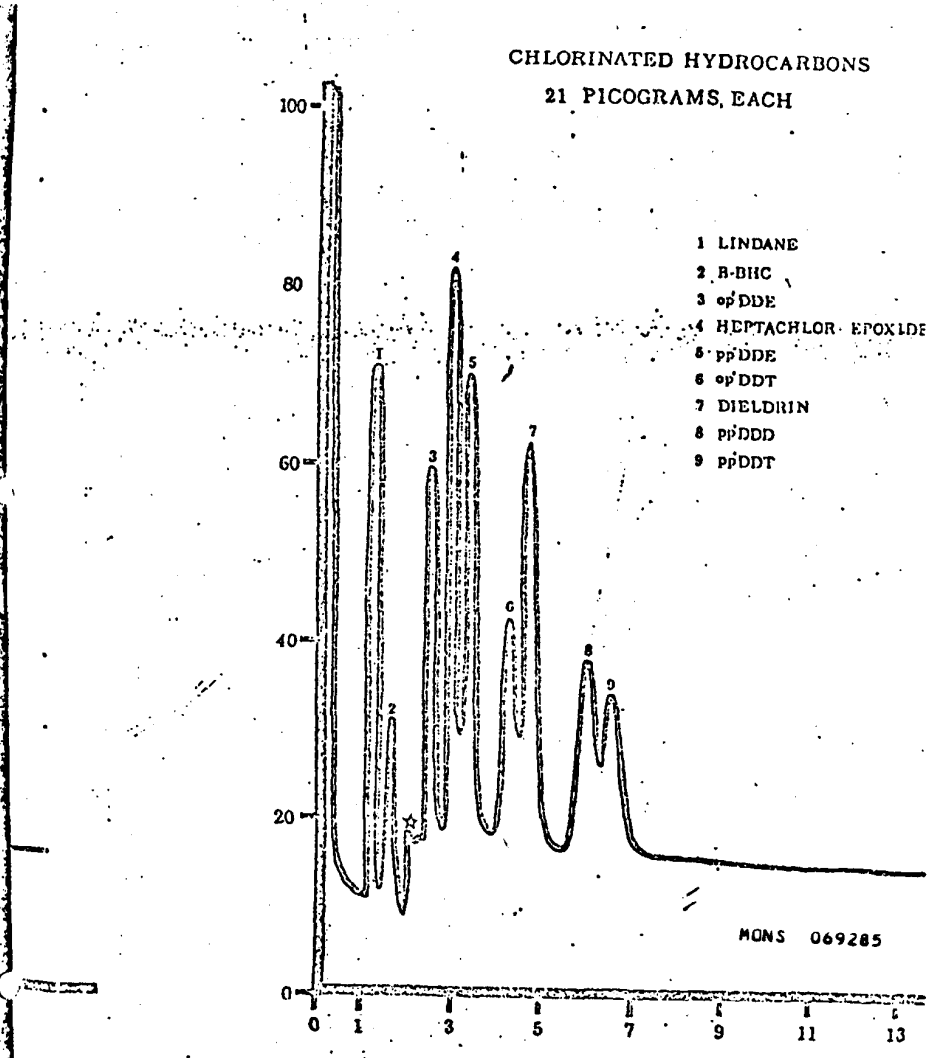
CHLORINATED HYDROCARBONS
21 PICOGRAMS, EACH
1 LINDANE
2 B-BHC
3 op'DDE
4 HEPTACHLOR EPOXIDE
5 pp'DDE
6 op'DDT
7 DIELDRIN
8 pp'DDD
9 pp'DDT
100
80
60
40
20
0
0 1 3 5 7 9 11 13
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Figure 2. STORAGE of PCB-Derived MATERIAL in TISSUES and PLASMA

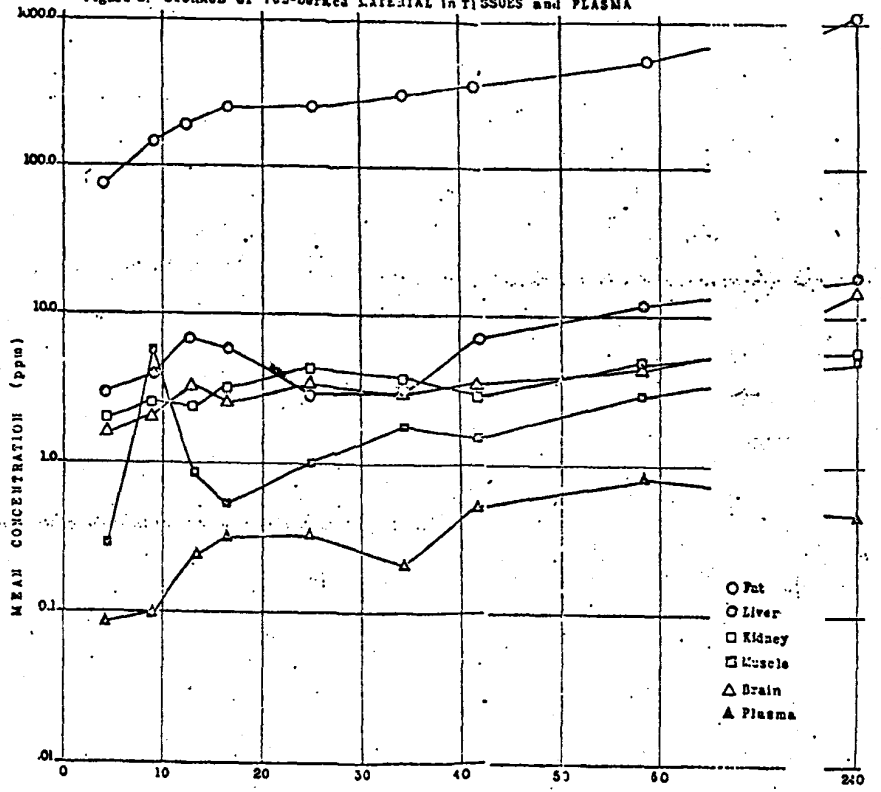
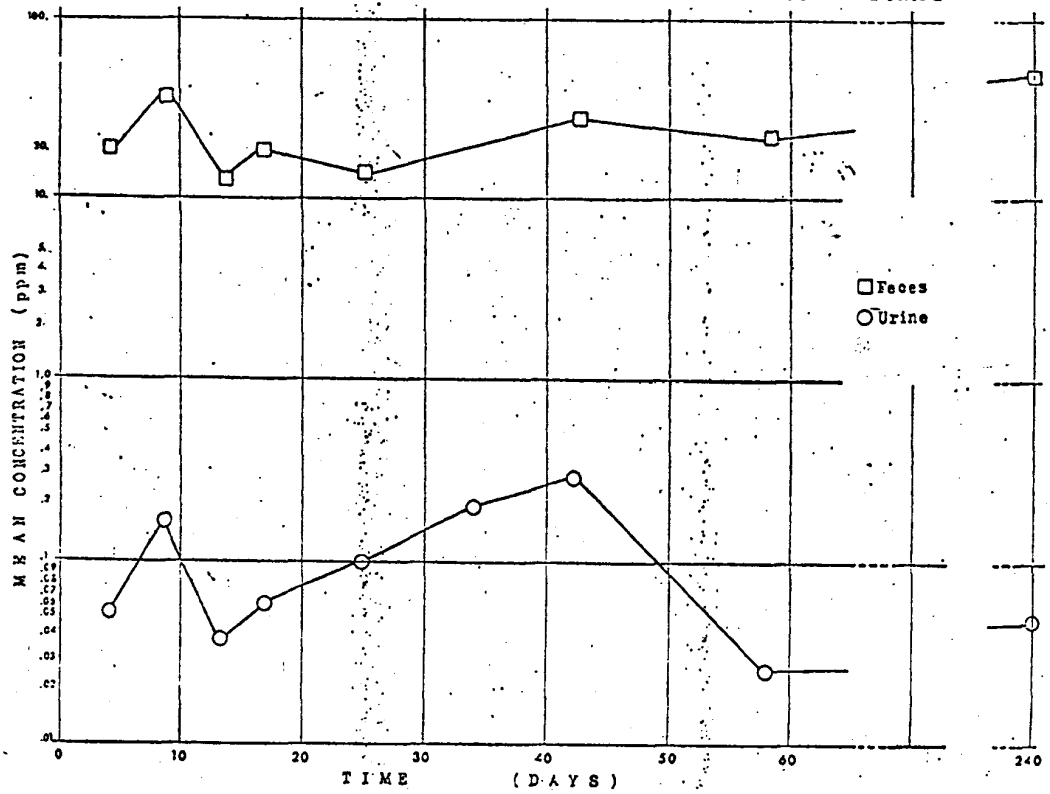


Figure 3. EXCRETION of PCB and PCB-Derived MATERIAL in FECES and URINE



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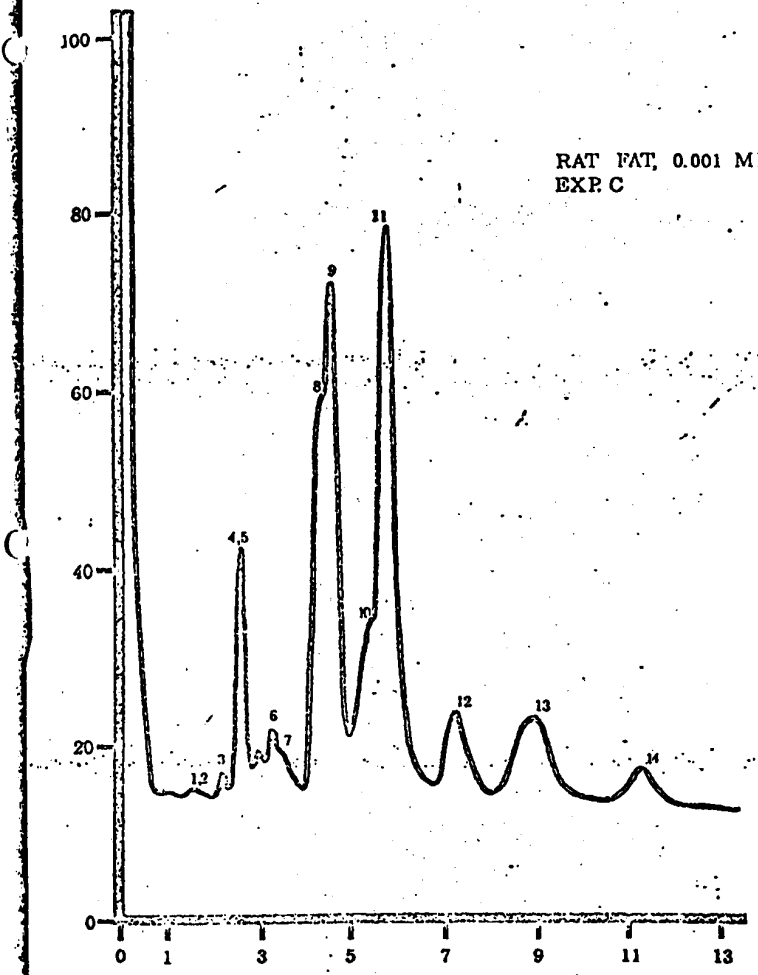
RAT FAT, 0.001 MILLIGRAM
EXP. C
100
80
60
40
20
0
0
1
3
5
7
9
11
13
1,2
3
4,5
6
7
8
9
10
11
12
13
14
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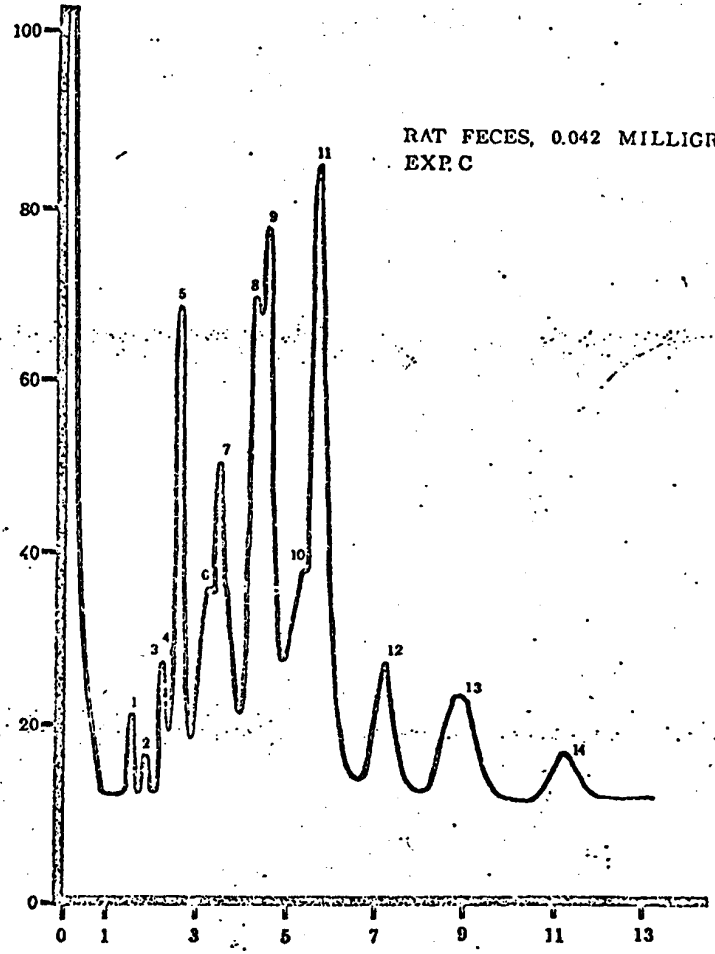
RAT FECES, 0.042 MILLIGRAM
EXP. C
100
80
60
40
20
0
0
1
3
5
7
9
11
13
1
2
3
4
5
6
7
8
9
10
11
12
13
14

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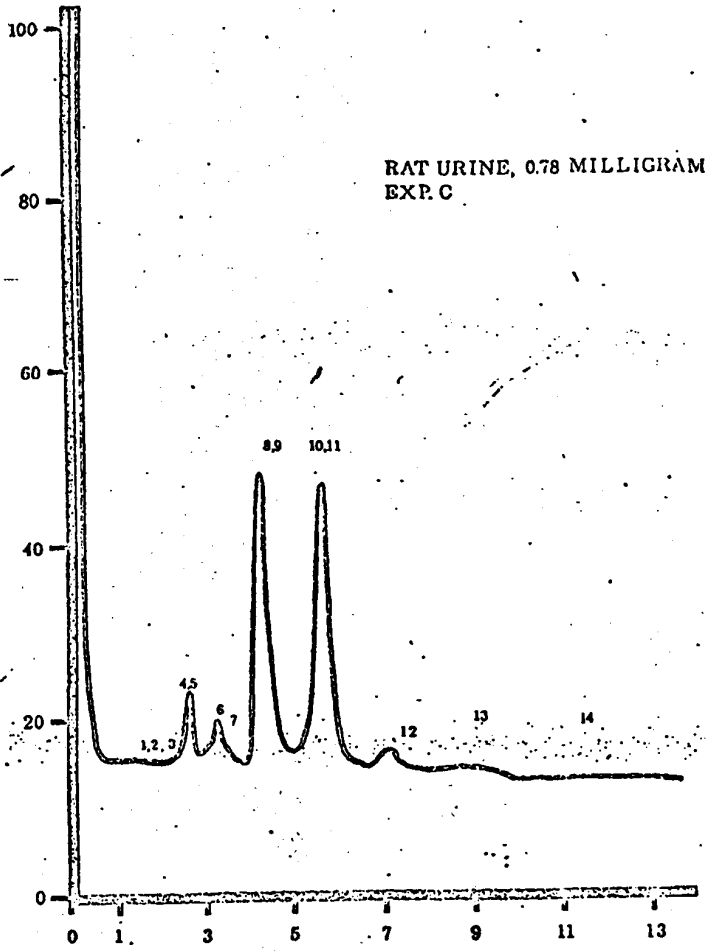
Fig. 4



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APPENDIX II

Experiments to date not included in Manuscript

Experiment No.	No. of Rats	Sex	Age at Onset of experiment (days)	Wt. (Gms)	dosage/route
Ar 7-70-B12	49 (analyzed)	F	30	224*	100 ppm Diet for 247 days * 8.1 mg/kg/day
Ar-7-70-B12	3 (analyzed)	F	30	224*	100 ppm in Diet for 300 days * 8.1 mg/kg/day
Ar-7-70-B12	3 (analyzed)	F	30	224*	100 ppm in Diet for 366 days * 8.1 mg/kg/day
Ar-7-70-B10	4 (analyzed)	F	30	240*	20 ppm in Diet for 280 days * 1.6 mg/kg/day
Ar-8-71-B	A study to determine placental transfer, excretion in milk and consequent tissue storage in weanling rats of Aroclor 1254 when the compound is given to the mother rat during organogenesis.				
Ar 7-70-C4	The effect of Aroclor 1260 upon reproduction in female rats when the compound is given during organogenesis.				

* Average weight of group and average rate of food consumption for group.

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Additional residue studies, including experimental design and resulting data, are listed on the following pages:

Experiment No.	Tissue	Mean ppm (4 animals)	± SE
Ar-7-70-B12	Fat	778	72.4
	Liver	9.9	1.16
	Kidney	5.1	.95
	Muscle	1.22	.104
	Brain	6.96	.68
	Plasma	1.6	.311

When compared to males at the same dosage level for an equal amount of time, Student's t-test shows significant difference in brain and liver storage. When these data are compared to data from male rats on the same dosage for an equal amount of time (see manuscript) the Student's t-test shows significant differences for brain and liver storage.

	Tissue	Mean ppm (3 animals)	± SE
Ar-7-70-B12	Fat	1022	127
	Liver	14.6	0.69
	Kidney	2.8	0.21
	Muscle	1.2	0.11
	Brain	9.3	0.57
	Plasma	2.1	0.43

When these data are compared with 8 mo. study test for significant difference shows $p < 0.05$ for liver only.

	Tissue	Mean ppm (3 animals)	± SE
Ar-7-70-B12	Fat	820	216
	Liver	22.5	2.51
	Kidney	4.7	.24
	Muscle	2.9	.96
	Brain	11.2	1.71
	Plasma	3.0	0.78

When these data are compared with 10 mos. study t-test for significances show $p < 0.05$ for liver and kidney.

	Tissue	Mean ppm (4 animals)	± SE
Ar-7-70-B10	Fat	274	48
	Liver	2.2	.10
	Muscle	.32	.06
	Kidney	.60	.05
	Brain	2.6	.50
	Plasma	.42	.06

The results of a study with Aroclor 1254(R) to determine placental transfer, rates of excretion in milk and consequent tissue distribution and storage levels in fetuses and weanling rats following oral dosage to the mother daily on the 7th through the 15th day of gestation at 10 mg/kg and 50 mg/kg, respectively by stomach tube is summarized as follows:

Fetus Analysis: Samples were taken by Caesarian section on the 20th day of pregnancy. The mean concentrations of Aroclor are given for the respective dosages as ppm.

	Controls	10 mg/kg	50 mg/kg
Mother 1	0	1.42	2.42
Mother 2	0	1.24	2.90
Mother 3	0	1.14	2.93

10 mg/kg

	Wt. at onset (gms)	Age (days)
Mother 1	254	90
Mother 2	265	90
Mother 3	258	90

50 mg/kg

Mother 1	242	90
Mother 2	244	90
Mother 3	261	90

Tissue Analysis: Samples were taken from 21 day-old male and female weanling rats. The concentrations in the tissues for three animals are given in ppm.

	Controls		10 mg/kg		50 mg/kg	
	Males	Females	Males	Females	Males	Females
Liver	<0.05	<0.05	4.06	2.85	17.36	21.63
			3.28	8.28	25.56	12.02
			2.70	4.11	11.80	15.99
		Mean $\bar{X}$	3.35	5.08	18.24	16.55
		S.E. $\pm$	0.39	1.64	4.00	2.79

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	Controls		10 mg/kg		50 mg/kg	
	Males	Females	Males	Females	Males	Females
Brain	<0.08	<0.08	1.96	1.94	10.16	10.48
			2.28	2.72	5.44	3.89
			2.24	1.24	7.47	6.75
		Mean $\bar{X}$	2.16	1.97	7.69	7.04
		S.E. ±	0.10	0.43	1.37	1.91
Kidney	<0.04	<0.04	1.52	1.54	7.53	2.41
			2.42	2.19	6.73	2.25
			1.14	2.32	6.70	4.10
		Mean $\bar{X}$	1.69	2.02	6.99	2.92
		S.E. ±	0.38	0.24	0.27	0.59

Milk Analysis: Milk samples were taken from suckling rats 11 days after the last dose to the mother. Concentrations are given in ppm.

	Controls	10 mg/kg	50 mg/kg
Range	0	16.48 -24.90	45.80-100.29
Mean	0	20.60	66.35
S.E.		± 1.59	± 8.36

Ar 7-70-C4

The results of a study with Ar 1260 show consequent tissue distribution and storage levels in weanling rats following oral dosage to the mother daily on the 7th through 15th day of gestation at 50 mg/kg by stomach tube is summarized as follows:

Note: One female and one male taken from each of 3 litters for analysis.

Tissue	Sex	Mean (3 animals)	± S.E.
Kidney	M	7.38	2.56
	F	5.44	1.41
Brain	M	7.53	1.04
	F	7.76	2.54
Liver	M	12.80	1.72
	F	16.13	6.75

Weights of Mothers 284, 264, and 288 gm

Mean weight of Mothers 278 grams