Notes from Trip of European T/P

ROUGH DRAFT - 6-12-69 dictated by R. E. Keller

April 28th: Mr. A. V. Holden, Dept. of Agriculture and Fisheries, Fresh Water Fisheries Laboratory, Pitlochry, Scotland. Mr. Holden is a very rational and understanding person on the question of pesticides and PCB's appearing as residues in wildlife. In fact, he responds more like a Monsanto employee than a government laboratory representative. He discussed very openly and willingly all details of his work and exhibited very cooperative attitude for future work of any kind that might develop either between ourselves and his laboratory or ourselves and other laboratories in the United Kingdom. The following pieces of random information were picked up during the course of our conversation with Mr. Holden.

When asked about the possibility of pesticide degradation and the production of chlorinated biphenyl products, he commented that there is evidence for DDT degradation but that it does not explain all the chlorinated products being found as interferences in work directed toward pesticide residue measurements. His prime concern has been from the interference standpoint of PCB's in measurements directed toward pesticides. He has not made an exhaustive, or for that matter, preliminary study of the type of interferences being found. His laboratory is one equipped quite well with gas chromatographic equipment but it has no equipment such as mass spectrometers needed for final identification of residues separated by gas chromatography. He commented that he could not use Jensen and Widmark's selective pesticide degradation procedure which they reported in their earlier work. The hexane partition system gives emulsions and he, instead, uses an alumina column which gets rid of fats. This is a column composed of 80 to 200 mesh alumina in 2 gram amounts in the columns. The silica gel dried at 120°C and to which 5% water has been added back has been used with hexane and hexane containing 10% ether to chromatograph residues containing pesticides. With this procedure the PCB's come out first and 8 to 10 pesticide peaks are also eluted in this manner but at a later date in the chromatograph. Tissue



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preparation involves taking one gram of tissue, 5 grams of anhydrous sodium sulfide, grinding ~~an~~ immorter in pestal ~~of~~ and extracting with a ~~sox of~~ soxhlet extractor for 30 minutes. He apparently obtains complete recovery and this has been demonstrated by spiking the tissue and carrying out recovery procedures. When asked how do we really check the recovery with biological tissue, he commented that radioactive DDT has been used to check the recovery, however, those doing the work could not pick up residual carbon$_{14}$ DDT and the results were not conclusive. The gas chromatographic procedure he commented on is one published by the FDA 3 to 5 years ago. The conditions are isothermal 200°C, electron capture, Chromasorb W 80 to 100 mesh, and acid washed. One column is coated with 10% DC 200 and a second column with 5% DC 200 and 7-1/2% QF-1. He commented the columns must have 1500 plates, must not break down DDT and must have no tailing of peaks. A nickel detector cannot be used because peaks overshoot making it difficult to read. No work has been done by Holden on thin layer chromatography.

Holden feels that an unacceptable level of DDT and Dieldrin in water is 1/1000 part per million. Experiments have been carried out where concentrations at this level have resulted in subsequent concentrations in fish at 1 part per million levels after six weeks exposure. At our levels, above 1 part per million, the fish died. This work was done using Rainbow trout as the fish. On this basis, the threshhold level for pesticides is 1/1000 of a part per million and with a 10X safety factor this becomes 1/10,000 of a part per million which would be a tolerable level regarding waters containing fish. The evidence showed that there was a steady build up of these pesticides in the tissue of the fish over the six week period. He did not know if lower concentrations for 12 months would be critical. He thought that work of this type had been done with PCB's, also in the UK government laboratories, but was not certain. At this point, he called London and the party on the other end said they would report back after they had searched the files. This call was not returned during the course of our visit and by arrangement with Hardy a follow-up will be made later to determine what was found at on

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this point. Holden commented that he felt that fish were more sensitive than other experimental species such as birds and mammals to toxicity studies of this type. When asked about where the PCB toxicity information was being obtained, he commented that Widmark, Jensen and others are referring back to the encyclopedia article xxxxxxxxxxxxxx prepared by Mr. Hubbard on chlorinated biphenyls several years ago. This is Volume 5 of this encyclopedia. This work, of course, contains no real information toxicity-wise that's of value at the levels and under the conditions of concern related to the PCB environmental contamination. He commented that the OECD of which Ao is the secretary, planned a conference in mid-September. This would likely be a closed conference where industrialists would not be invited. His proposition for this meeting was to take birds and fish and show two levels of residues which could be considered as normal and abnormal and present this to the biologists and ask to show a meaningful difference between the levels and on this basis set a residue level. He feels that we just can't accumulate data and not know the significance. He recognizes the complexity of the problem and how to get meaningful concentration levels but emphasizes that the biological consequences to the real world situation must be clarified to know the significance of the residues being found. It will be interesting to see if there is really a proposition of this type and resulting follow-through coming out of the September meeting.

The following three reprints of publications were given to us upon our departure:

1. Cleaning of Tritium Foil Electron Capture Detectors published in the Journal of Gas Chromatography, July 1967.
2. The Examination of Surface Waters and xxxxx Sewage Effulence for Organo Chlorine Pesticides, published in the Journal and Proceedings of the Institute of Sewage Purification, Part 3, 1966.
3. Organo Pesticides in Seals and Porpoises published in Nature, Volume 216, No. 5122, pp 1274-1276, 1967.

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April 29: Tunstall Laboratories, Shell Research, Ltd., Sittingbourne, Kent. Those present for Shell were Dr. J. Robinson, biologist, Mr. A. Richardson, analyst, Dr. Stevenson in charge of experimental animal house, Mr. DeCorsen, laboratory administrator - and for Monsanto, R. E. Keller, E. P. Wheeler and D.V.N. Hardy of NCL. Shell commented that their analytical work was, and a part of it is still, based on work reported by Eidelman, M., in the Journal of Association of Official Agriculture 46 182 (1963) with Open Tubular (Golay) Column" ~~chlorinated (Goulet)~~ (column) in Perkin-Elmer Application No. GC-PS-011. (Another reference was "Analysis of Chlorinated Biphenyls" Shell's work was directed toward the pesticide problem in its early stages. Later they ran into unexplained interferences and it wasn't until the work of Widmark and Jensen that they really knew the identity of these interferences. Their work had been concentrated, for example, on the analysis of Heron eggs and oils, using the gas chromatography electron capture procedure. During the course of this work, they ~~have~~ found a higher concentration of PCB's than pesticides. They commented that the government laboratories in the UK have found as high as 900 parts per million PCB's in such products. They also commented that the use of one million gallons of high pressure oil as lubricant is used in the UK per year and this may explain ~~our~~ high concentrations of this material compared to the pesticides found. About 3 to 4% of this total volume of high pressure oil would be PCB related material. They also mentioned that one thousand tons of DDT have been used in Kent since the war. The main DDT usage being on fruit. Shell has confirmed 8 to 12 peaks found by gas chromatography as PCB's and more specifically as those related to our Aroclor 1254.

Shell used ~~raising~~ racing pigeons to carry out toxicity studies. Results show no change where 10 to 12 parts per million Dieldrin were found in eggs. Other feeding studies involved feeding 100 parts per million of Aroclor 1254 to chickens for 1 year with no mortality and

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with no effect on egg laying. Also shell pigment of the egg was not effected. A missing link to this type of study was the PCB residue levels related to the studies and this should be determined in the future. Shell has a very well equipped laboratory with an MS MF-9 mass spectrometer and personnel who are highly competent and very capable of carrying out studies of the type of Widmark and Jensen. They were very cooperative in their discussions and left the door open for future follow through, whatever it might be on this common problem. Their interest, of course, is primarily insecticide related and not from PCB viewpoint. Shell provided 3 bar graphs of mass spectrum for Aroclor 1254. In addition, the following publications were provided: a working paper on Mass Spectrometry of Organol Chlorine Compounds and Attempts to Reach Positive Identification which was read before the [illegible] meeting by [illegible] Widmark in October, 1968 at the International Union of Pure and Applied Chemistry, Applied Chemistry Division, Pesticide Section, Commission on Pesticide Residue Analysis. The second paper is entitled, Some Recent Analytical Developments in Respect to Organo Chlorine Compounds in the Environment, by A. Richardson, Shell Research, Ltd., Tunstall Laboratories, Sittingbourne, Kent, England.

May 1: Laboratory of the Government Chemist, Monsanto House, London, England. Mr. J. O. G. Patton and G. B. Collins of the Laboratory of the Government Chemist, and Messrs. R. E. Keller, E. P. Wheeler and D.V.N. Hardy met and discussed the problem of PCB and pesticide residues. Mr. Patton reviewed the total problem as they had experienced it starting in 1953 when it had been noted that PCB compounds were being used in food packaging in government annual reports. In 1963, pesticide GC work in the governmental laboratories showed peaks which were not organochlorine pesticides related and this was also found in wildlife analyses. At this time they were not sure if the peaks being observed in gas chromatographic techniques were the result of breakdown products. Jensen in Sweden pointed this out in his report mentioned earlier. Overall, the PCB has been found in wildlife, especially aquatic wildlife, and eggs. It has apparently not been detected in foods and only rarely at very low

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levels in human fat. The detection limit is 0.01 parts per million PCB. The amount found in Britain in human fat is 2 parts per million and in the United States 8 parts per million, most of this being pesticide or DDT related. Traces of PCB have been found in the human lung. 1×10^{-12} parts per MMKKMXM million DDT has been found in the air. For clarification, American notation of parts per billion is 10 to the 9th, and the British notation is 10 to the 12th. Patton commented that there is a very high DDT content in the London air and this is apparently the result of the pesticide sticking to carbon in the area.

Patton was curious about what PCB's are used for in Britain and about how they get to birds since they apparently don't get to man or domestic animals. Again, it was mentioned that the Heron liver contains 200 parts per million PCB. He commented and qualified the statement that this level cannot be estimated accurately. The Natural ~~Natural~~ Conservatory in the UK sends liver, eggs and brain tissues to Patton for analysis. PCB peaks in chromatograms from bird tissue represent only a portion of those found. He felt that he could not comment on the entire chlorinated species simply because he didn't know what these might be. The question about Concord jet aircraft blowing out chlorinated biphenyls was brought up and Monsanto was asked about what we know on this point. To our knowledge, no uses of this type exist except for a very nominal amount. Patton has found PCB MMM in terrestrial hawk type birds as well as sea birds. Chemical degradation of pesticides have been carried out in their laboratories and they could not produce GC chromatograms of the type related to PCB compounds. Again, Aroclor 1254 was associated with the type of chlorinated residues being found. Traces of PCB have been found in milk and in beef fat. Patton knows of no one else in the UK other than Shell and Holden who are working on the PCB or pesticide PCB related problem.

A significant part of the conversation revolved around the point that residues from PCB are higher in aquatic birds and fish than domestic animals and humans. No PCB's have been found in the river waters of the UK. This detection would be at parts per 1/1000 million level.

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Patton will send annual/reports to Dr. Hardy at MCL who in turn will forward pertinent information back to the States. Again, they are concerned primarily with intereferences and not with measurement of PCB. It was obvious that a coordinated project has not yet evolved in government laboratories where the source of tissues in birds could or would be correlated with residue amounts. However, it is safe to assume that in time a special effort will be made to provide follow through on a program of this type in the government laboratories. It was agreed that we would be happy to provide GC data for Patton, et al, as we develop this in our laboratories. In conclusion, while this work has been very informative relative to the gas chromatographic separations, it is not worth involving positive identification through mass spectroscopy as has been carried out by Widmark.

May 5: Institute of Analytical Chemistry, University of Stockholm, Stockholm 50, Sweden. Messrs. Keller, Wheeler and Digby of Monsanto and Richard of Monsanto met with Prof. Gunnar Widmark and discussed the problem of PCB's and the environment. Widmark, while basically ann analytical chemist, has considerable know-how about the total problem related to environmental contamination and the ecological system in general. Widmark is a rational person who exercises great caution in work that is done and published. He has regretted that the course has gone the way it has resulting from publication of their work. He commented initially that his colleague, Jensen, was no longer with him or a part of the Institute of Analytical Chemistry. Jensen subsequently could not be located as he was out of town and not available. It is our understanding that he will become a participant either in academic or government related coordinated projects more from the viewpoint of ecological system involvement as it relates to PCB. The following comments cover our discussions with Widmark today, May 5, and subsequently by R. E. Keller with Widmark on May 7.

Widmark surveyed the development of events leading to their work in publications involving PCB. In 1964, they had grants for pesticides

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and Jensen who was with the Institute of Analytical Chemistry, started working on the problem at this time. During the course of this investigation, they found interferences which in turn led to additional studies resulting in the identification of PCB's by mass spectroscopy. Out of this came the now famous lecture referred to by many in the States and in the UK and Europe as the first insight as to the identity of interfering components. Widmark was not eager to have these results published by Jensen at this time. At the end of March 1969, there was a meeting in Stockholm of most of the people presumably in Sweden doing analytical work. It was found that DDT, DDE and PCB's were increasing in concentrations in fish and aquatic wildlife. Widmark refused to sign any endorsement or recommendation out of this meeting. His position being that it is very difficult to quantify the PCB results because of the many isomeric species, and that we really don't know which group we are working with under these conditions. Furthermore, at this time, the metamollic processes in terms of stability of PCB type compounds was not known. The comment was made that Holland (?) fish contained less PCB than those in Sweden. Chlorinated naphthenes are not found. Naphthenes are probably metabolized in a manner similar to PCY's. About 2,000 to 3,000 samples, presumably wildlife, have been analyzed in the Stockholm laboratory of Widmark. In his opinion, this is not a sufficient sampling to provide a summary of the information obtained. His theory on findings observed is that the 5, 6, and 8 chlorinated biphenyls are not metabolized because not many below 5 are found. For his work, the German chlorinated 50% biphenyl was used as a reference standard. In figure 5, peak 3 diminishes through the food chain whereas peak 10 related to 5 chlorines increases. This is evidence for metabolism. Toxichene treated with sulfuric acid does not give PCB's. American eagle residues gave PCB chromatograms after sulfuric acid treatment that were different than reference German chlorinated biphenyl compounds. Dr. Stickles, at the Pawtuxet Laboratory in America, who is our representative with the OECD group is participating in a joint analytical program with Widmark. Apparently, 5 samples have been circulated to 12 laboratories. No common method has been circulated such as for round robin testing

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but rather each laboratory is to use its own procedure and make determination of PCB's and presumably pesticides in these common samples. Representative portions of homogenized samples were sent to each laboratory. One objective of this exchange is to determine analytical difficulties between laboratories and different procedures. Results are to be compared in about six months at a September meeting of the participants. One objective out of this work is to build a background of information about PCB's in residues in birds and fish in non-industrial areas. A worldwide picture will be obtained to form a basis for future action. To date, no real toxilogical work has been done in Widmark's opinion which he, at least, can understand. Only a very few toxicity studies are being done currently in Stockholm. In his opinion, too little is being done. He believes that there is little hope that analytical and toxilogical activities in Sweden will be combined. He believes that the OECD group is not really considering toxicological results and that this will follow later. The question of composition of exit gases from automobiles was brought up and discussed briefly. There is apparently no evidence for degradation of pentachlorophenol or hexachlorophenyl compounds. Widmark takes the position that we must know where the PCB compounds come from and more about the toxilogical effects. He apparently has not been impressed in his words by the analytical ability of Risebrough at the University of California. He thinks that society will ask for biodegradable compounds of the type we provide as Aroclors in the future. Widmark has work planned related to repetitive mass scanning with a CAT for more sensitive and perhaps specific determination of isomeric species being found in residues. Differences in Monsanto Aroclors and those manufactured in the UK and Europe are not sufficient to permit identification using mass spectroscopy techniques. Widmark agreed to send GC chromatographs of Monsanto and competitive product. He promotes the dropping of PCB as a product and in turn the offering of a substitute product. He thinks it is expensive to support the position we may have to take or prove or disprove the use of Aroclors. PCB users, presumably those involved mostly with transformers, in Sweden were contacted. Most replied they had one accident with one man in which the fat and blood was checked. The results from this apparently

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were not conclusive. No further work was done involving humans primarily because government funds were not available. When asked what he would do if he were in a manufacturer's position involving PCB, Widmark commented as follows:

1. Restrict PCB's to closed systems.
2. Find a substitute for PCB.
3. Come up with a positive analytical data so that biologists ecologists cannot misuse it.

Widmark provided the following reprints of publications for our reference: "Determination of the Number of Compounds which can Result from the Chlorination of Biphenyl and Development of a Simple System by which these may be Codified," "Methods of Analysis and Examples of Chromatograms, the OECD study of analysis of PCB, December 1968 and "Results Obtained by Analysis of the OECD Exchange Samples 1, 3 and 4." Our methods that we either are using or plan to use for the gas chromatographic work and mass spectrometry work were reviewed with Prof. Widmark. Comments from these discussions are noted directly on the reference attached copies of the individual procedures. He feels that the chromatographic and mass chromatographic procedures should be similar for direct comparison as opposed to having separate conditions such as isothermal vs. programmed temperature separations.

May 9: Professor H. vanGenderen, Veterinary Pharmacology and Toxicology, University of Utrecht, Biltstraat 172, Utrecht (Netherlands). Messrs. Wheeler and Keller discussed with Prof. vanGendern and Drs. J. H. Koeman, biologist, W. L. van der Maas, chemist and T. G. Vos, veterinary pathologist, the problem of PCB and environmental contamination. Prof. vanGenderen is concerned with pollution of the environment at the scientific level and not at political levels. He is chairman of one of the pesticide pollution committees in the Netherlands and acts as an advisor to the public health institute. He teaches pharmacology at the university, has contact with biologists and uses students on PhD projects related to biological toxicological studies. He tries to involve students with problems of society as well as scientific

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direction only. The problem of the diminishing birds and wildlife species has been of concern with the thought that this may be caused by poisoning. Chlorinated hydrocarbons, mercury compounds and perhaps polychlorinated biphenyls are contributors to the demise of certain species. For this reason, they embarked on a program to make a study of fish and birds from the Netherlands area and the resulting work was published in Nature ~~xxxx~~ entitled "Chlorinated Biphenyls in Fish, Mussels, and Birds from the River Rhine and the Netherlands Coastal Area", the reference being Nature, Volume 221, pp 1126, March 22, 1969. Monsanto and European products have been used as reference PCB material. No work has been done on domestic animals and humans in terms of analysis for residues. It will be only a matter of time until human~~s~~ studies of this type are made according to vanGenderen. The highest residue levels appear in birds and the higher figures are related to the Rhine as compared to the sea. On this basis, vanGenderen believes that the contamination dateline(?) is coming from Germany. No work is underway on the concentration of PCB's in the water of the Rhine. He believes that it is only the lower chlorinated PCB's that escape from the water. The PCB's are higher in aquatic life than pesticides. Their toxicity studies indicate that hexachlorobenzene is worse than European PCB's and toxicity studies. There has been no work with other pesticides. The mass spectroscopy work for their studies is carried out at a separate laboratory on a contract basis; a facility which is similar to the Battelle Institute in the United States. The mass spectrometer is a Varian Atlas CH4 instrument. Upon questionning the members of this group more closely about the mass work, it became apparent that this work was done elsewhere. At this point, vanGenderen and the others volunteered to take us to deBrauw, who actually did this work. After visiting with deBrauw, it became apparent that they have all the expertise and gear needed to do a completely competent job such as that by Widmark and Jensen. deBrauw is an expert in mass spectroscopy having installed 3 or 4 instruments in the States for the Varian Atlas Company. Comments from discussion with him are noted on the attached copy of our method write-up and will be recorded in this manner.

Notes on other parts of this trip are reported in call reports by J. Hagyard and a special report by Hardy.

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