11-2-69

FF
Aroclor: Wildlife

S. Tucker's report to W. Richards

The analytical support work for the Aroclor-Wildlife project can be conveniently divided in to four areas: ① Develop sensitive and specific in hand methods for identifying Aroclors in our environment, ② Initiate an outside toxicological test program and provide the necessary analytical ~~by~~ methodology, ③ Determine the validity of the methods used and the results obtained by workers in the residue area, and ④ Monitor the ~~laboratories~~ laboratories doing research on environmental contamination by Aroclors.

The following progress has been made in the ~~all~~ above areas:

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① An F&M 402 high efficiency gas chromatograph has been purchased and set up. This system allows two detectors



operation and a choice of three detector systems, electron capture, flame ionization and microcoulometric. The GC-electron capture system is operational ~~has been completely~~ and has been completely evaluated with regards to Aroclor sensitivity and potential pesticide interferences. The second column (Dc-200 - Anakrom ABS) will utilize both the flame ionization and microcoulometric using a QF-1 - Chromsorb W column via an infinitely variable effluent splitter detectors. The electron capture system ~~will~~ provides an extremely sensitive method for identifying nanogram amounts of PCB and possibly a semi quantitative estimation of the amount present. The FID-microcoulometric system while not as sensitive should provide a better quantitative measurement. This second system is not operational because of electronic failures and has not been completely evaluated. The characteristics of the PCB's on two different

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columns and three detectors will provide for their ~~an~~ almost ~~an~~ unequivocable identification.

In addition to this work Dr. Mieure has ~~[illegible]~~ established the ~~necessary~~ operating parameters necessary for the ~~[illegible]~~ absolute identification of ~~[illegible]~~ PCB's by ~~[illegible]~~ GC-Mass. ~~spect~~ ~~[illegible]~~

② An outside toxicity study has been initiated with Industrial Bio-Test Laboratories and the analytical support work necessary completely outlined on paper. Some tissue samples are ~~[illegible]~~ now in hand and verification of the outlined procedures will begin soon.

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An alternate hot analytical approach using a 19 traced Aroclor has also been

outlined on paper and is available. Should its use be necessary.

③ After closely reviewing the available literature I find it difficult to dispute the identification of the interfering peaks as PCBs. Still we have not ~~actually~~ demonstrated this on ~~actual~~ real samples in our laboratories. For this reason a letter to the Fish & Wildlife people at Patuxent is being ~~[illegible]~~ drafted requesting duplicate samples of some of the tissues in which they believe they ~~[illegible]~~ ~~[illegible]~~ ~~[illegible]~~ ~~[illegible]~~ ~~[illegible]~~ ~~[illegible]~~ found ~~peaks~~ PCB's. These samples will then be subjected to an ~~[illegible]~~ absolute ~~[illegible]~~ GC/MMS identification. If PCB's are found this still doesn't not prove

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that our Aroclors are the ~~initial~~ source of contamination. To help us determine if aroclors are the actual source of ~~this respect~~ the following cont. experiments have been planned.

1. Water solubility of Aroclors
2. Incineration ~~[illegible]~~ of Aroclor containing paper
3. Analysis of ~~environmental samples~~ - air, water, & soil samples ~~especially in the [illegible] of~~ from around our manufacturing sites.
4. Feeding studies with pesticides that might be metabolized to PCB's

(4) So far we have visited several government agencies around Washington to evaluate their interest. Other trips will be planned as deemed necessary.

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Electrical Fluids - Air & Water Pollution Control

	Project	EW No.	Est. effort	Priority
(1)	Develop and evaluate GC/GC and GC/MC with various columns for Aroclor analysis.	1	1-2 wks	1
(2)	Develop methods for Aroclor analysis of rat muscle, fat liver, and kidney samples	2 & 6	3-9 wks	4
(3)	Develop methods for Aroclor analysis of chicken muscle, fat, liver, kidney, and egg samples and feces	1	2-3 wks	2
(4)	Develop methods for Aroclor analysis of duck muscle, fat, liver, and kidney samples		2-3 wks	5
(5)	Verification of Potapaug and Rosebrough GC identification of PCB by GC/mass	8	1 wk	1
(6)	Incineration expts with Aroclor containing paper	1	1 wk	3
(7)	Aroclor water solubility study		1-2 wks	6
(8)	Pyrene water solubility study		4-6 wks	8
(9)	PCB analysis of environmental residual samples: air, water, soil, & wildlife		4-6 wks	7
(10)	Write up and compile data on C^{14}	5	1-2 wks	0

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Aroclor - Wildlife

Proposed Research - General Outline

The proposed research can be guided by two basic analytical needs. First a need for sensitive and specific methods to analyze environmental samples for polychlorinated biphenyls (PCBs) in the presence of other chlorinated pollutants, and secondly to analyze biological samples from test animals used to establish the short and long term toxic effects of Aroclor.

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The most logical manner in developing methodology of this sort is to start at the finish and work backwards. Guided by this and the basic needs of the project an F&M model 402 high efficiency gas chromatograph was purchased. This system when completely set up will allow the use of two columns and three detectors. The two

columns to be used are, a 6% QF-1 on 80/90 mesh Chromosorb W (polar liquid phase) and a 10% DC-200 (12,500 cs) on 70/80 mesh Anakrom ABS (non-polar liquid phase). A Dohrmann microcoulometric detector (DMD) to be used with the DC-200 column and via an effluent splitter the components eluting from the QF-1 column will be detected by both a flame ionization detector (FID) and a high temperature Ni^{63} electron capture detector (ECD).

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The equipment and mode of operation provides ~~a~~ a number of advantages and a great deal of versatility. The use of polar and non-polar columns gives us a system in which identical retention times for two different compounds are highly improbable. Also provides us with greater versatility in operating the PCB's

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from suspected interferences. The ECD is the ultimate with respect to (WRT) sensitivity for halogenated compounds. With this detector 1 nanogram of Aroclor 1242 can readily be detected. While possessing extreme sensitivity it has the disadvantages of not being entirely specific and of having widely varying response factors dependent upon both the nature of the functional group and its position in the molecule WRT other atoms. To compensate for this the DMD will be used on the SC-200 column. It allows halogenated organic compounds to be selectively detected and quantification is easily carried out. The FID is a general detector which is sensitive to all organic molecules yielding a more complete picture of the sample. In addition to this the sensitivity limits of

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the FID are directly transferable to our GC/MS system.

The ECD system has been evaluated via fingerprinting the various fractions and the retention times of the available PCB isomers and more common pesticides. The FID has been used but the splitter has not been installed or used on this column. The DMD is nearing but complete evaluation awaits elimination of electronic problems.

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The next step in the outline is the concentration of the fractions after being extracted from the samples. This step has been worked out using vacuum rotovary evaporator and Kuder-Danish evaporative concentrators. Overall recoveries on the order of 98-107% were achieved in the 250-2000 ppb range and there is no reason to believe that the lower ones

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be picked still lower, say by a factor of 10 or more.

The final step is the extraction and isolation of the steroids from the actual matrices and potential interferences which may or may not be present. The following types of sample matrices are anticipated: water, and also adipose fat, muscle, kidney, liver, urine, and feces. General procedures for the work up are outlined below, with the exception of urine and feces in this study since their inclusion was not required and no literature workup has been performed. However, I do not imagine that the workups will vary much from the ones outlined. No work has been carried out on actual urine samples. This will be started as soon as the various tissues are received from Biotest Laboratories.

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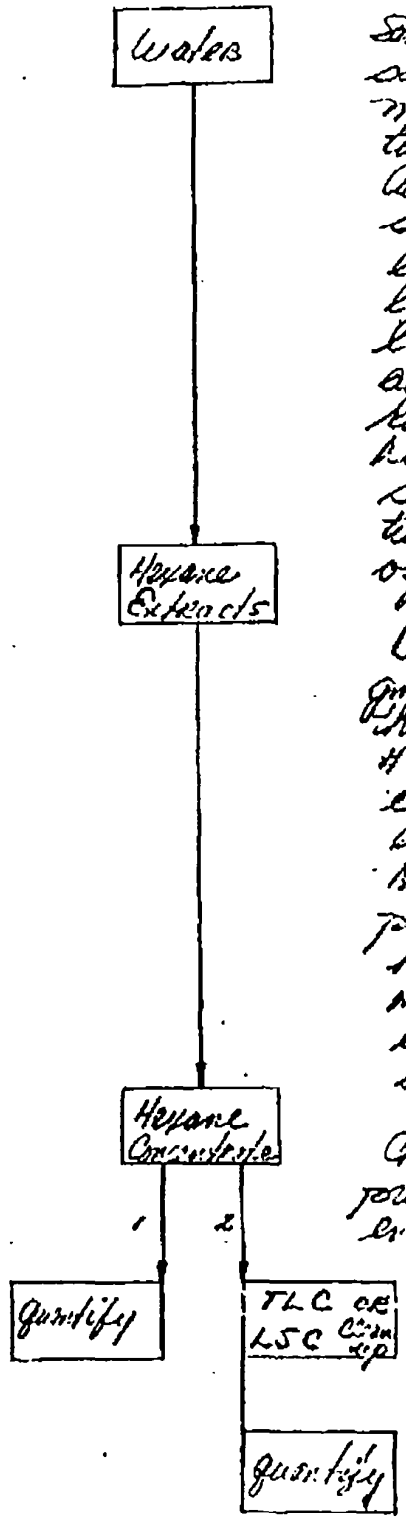
Water



Sample size 250-1000 ml - filter if sediment present. Transfer a measured volume of the sample to an apropriate size sep. funnel. Add 50 ml hexane to sep. fun. and shake a min. of 1 minute. Allow layers to separate transfer aq. layer to one beaker and hexane layer to another. Transfer aq. layer back to sep funnel, rinse beaker with 50 ml hexane and add hexane to sep funnel. Repeat extraction twice more with 50 ml portions of hexane.

Dry hexane extracts by adding 20 gm. anhyd. Na_2SO_4. Filter hexane through hexane-washed Whatman #2 filter paper collecting the extracts in a Kundra-Danish evaporative concentrator. Wash Na_2SO_4 and filter with 3-25 ml portions of hexane. Evaporate hexane, by placing concentrator in a boiling water bath, to less than 5 ml. Cool and dilute to exactly 5 ml.

Check at this point by EC/GC for presence of Aroclor and/or interferences

① Aroclor present no interference
② " " with "

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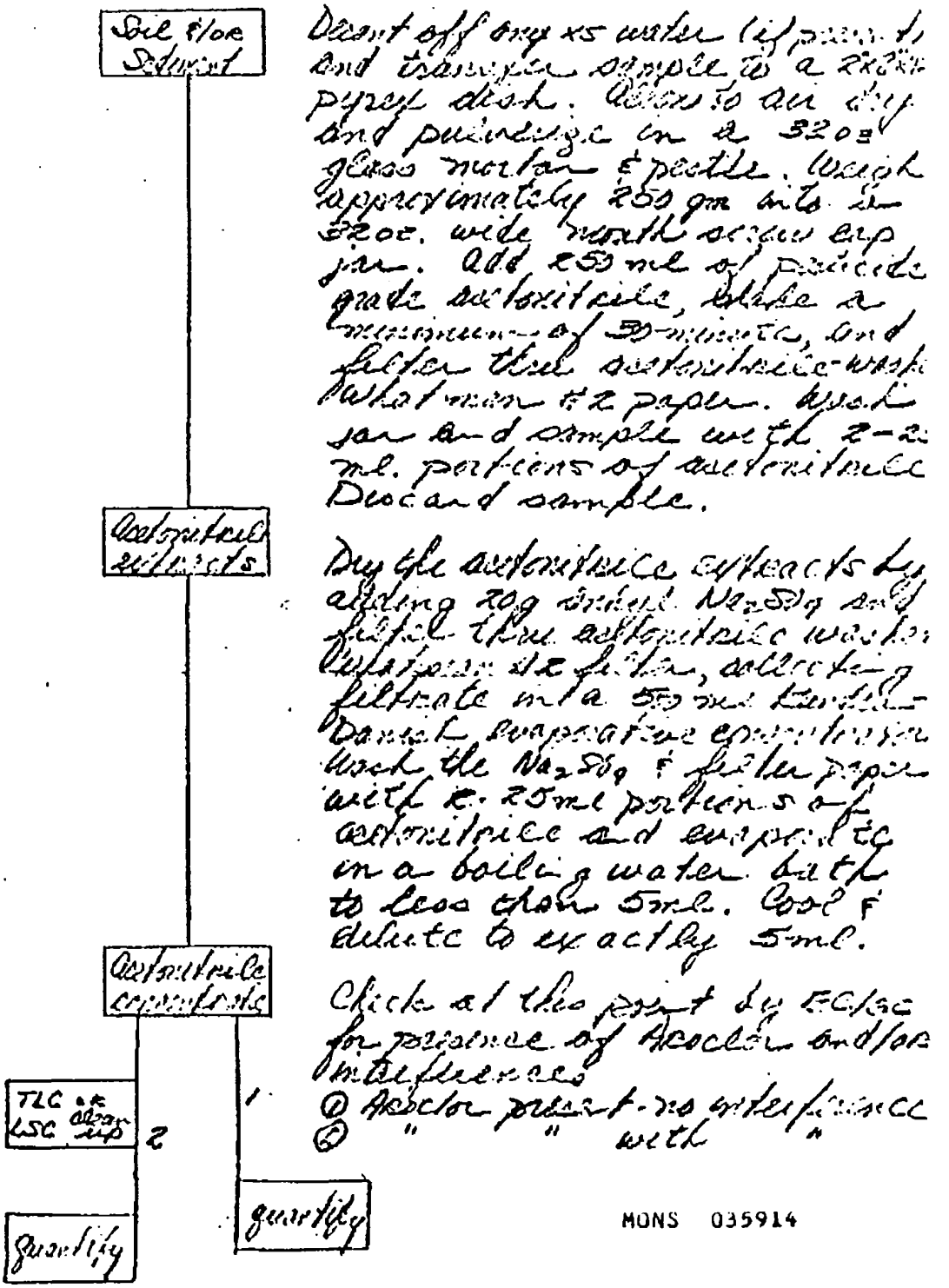
Sil f/or Solvent V.



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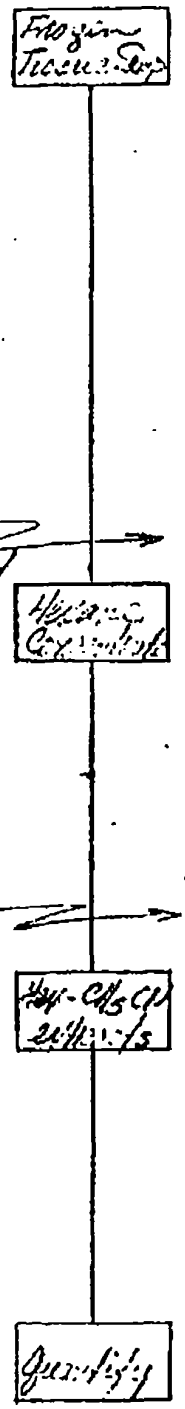
Muscle, liver, kidney, & fat*



Frozen Tissue Sump

Cut frozen tissue into 1cm sq. pieces and homogenize with 100ml hexane. Decant off hexane and repeat this procedure twice more. Filter combined extracts thru hexane-washed Whatman #2 filter paper. Wash beaker and filter paper with two 50ml portions of hexane. Combine hexane extracts & washes in a 500ml Kundera-Danish evaporative concentrator and concentrate to exactly 5ml.

Extracts may be completely evaporated & residue weighed to determine amount of fat present.

Hexane Concentrate

Transfer the hexane concentrate to a 125ml sep funnel - keeping the total volume at 25ml - and extract with 4-25ml portions of hexane saturated acetonitrile. Combine acetonitrile extracts and evaporate to dryn. Take residue up in 10ml hexane.

Muscle extracts are usually pure enough at this point for ECGC.

Hex-CH_3CN extracts

Transfer hexane to a 2x15cm column of 80/200 mesh Alumina (1-1.5% water) topped with a small layer of anhyd Na_2SO_4. Elude with 9:1 (V/V) hexane ethyl ether. Concentrate eluent to desired volume in a Kundera-Danish apparatus.

Quantify

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Water Solubility of Aroclors & Pydrauls

On August 20, 1968 a request from Lou Stark to determine the water solubilities of Aroclors and Pydrauls was received. The following work has been carried out ~~initiated~~ on this project. A procedure for extracting ~~and~~ concentrating and measuring Aroclors at low levels in water has been worked out and verified with spiked tap water samples (TABLE I). The following technique was used to prepare tap water saturated with Aroclor 1242, 1248, & 1254: Two 60-70gm samples of each Aroclor were placed in 32oz narrow neck screw top jars and 500 ml tap water added. ~~Records~~. The jars were then placed on a roller mill rolled for 24 hours and allowed to stand undisturbed at room temperature since 9-9-68. Recently (date of report) two 225-250 ml samples of the tap water, saturated with Aroclor

1242, 1248, & 1254 were analyzed using the outline hexane extraction EC/GC method. It is evident from the chromatograms that the isomer distribution of the hexane extract was different than that of the original Aroclors. It appears that the solubility of PCBs is a function of both the amount of chlorination and the position. This anticipated behavior makes the EC/GC difficult to quantify since a standard with the same isomer distribution is difficult to come by. The amount of Aroclor in the sample was therefore calculated in this manner: using a hexane solution of the original Aroclor a calibration curve was prepared by plotting the total peak height versus ug Aroclor. The peak heights of the same peaks in the sample were then totaled and the amount of Aroclor present determined from the calib curve. A rough idea of the type error involved in this procedure can be obtained by using 1242 as a standard

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for 1254 etc. The results of the solubility study and the error estimation are shown in Table II. & III respectively.

I had been hoped that the GH/GC would be working at this point so that a total chlorine figure could be used to estimate the amount of Aroclor present. Unfortunately it is still not operational.

No work on the Pyranol solubility study will be started until the Aroclor solubility study is completed

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TABLE I

Recovery of Aroclor from Spiked Tap Water Samples** Using EC/GC.

Sample Number	ppb Aroclor ADDED	Sample Volume (ml)	ppb Aroclor FOUND	Per cent Recovery	Comments
OR 86932 #1	1980	250	1960	99.0	Aroclor 1242 Samples contaminated with voc. sol. evap. Results obtained 9-6-68.
OR 86932 #2	1980	250	——*	——	
OR 86915 #1	270	225	266	98.5	Aroclor 1254 Samples contaminated Kuderna Danish evaporative concentrators - Results obtained 12-15-68
OR 86915 #2	270	225	266	98.5	
OR 86915 #3	540	225	577	107.0	
OR 86915 #4	540	225	577	107.0	

The sample preparation and work up outlined for water samples was employed. The results were quantified by preparing a calibration curve using a known solution of the Aroclor 1242 and 1254 used to spike the water samples. The major common peak area was used to construct the curve. The water samples were spiked by injecting aliquots of concentrated acetone Aroclor solution

* Sample contaminated - results discarded

** Data used to confirm proposed water sample work up procedure and concentration techniques

*** Spiked water samples contained 90-60 ppm Aroclor.

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Table II

Solubility of Aroclor in Tap Water

Sample Number	Sample Dry Wt (g)	Sample Volume (ml)	ppb Aroclor Found	Average	Comments
1242					
OR88936*-1	67.5	225			
OR88936†-1	67.5	250	413		
OR88936*-2	55.1	225			
OR88936†-2	55.1	250	421		
1248					
OR88936-1	70.5	250			
OR88936†-1	70.5	224			
OR88936*-2	70.6	250			
OR88936†-2	70.6	225			
1254					
OR88936*-1	73.2	2			
OR88936†-1	73.2	2			
OR88936*-2	69.7	2			
OR88936†-2	69.7	2			

* Aqueous layer filtered thru pre-wetted Whatman #2 filter paper

† Aqueous layer carefully siphoned from bottle.

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