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Report on the Technical Review Workshop on the Reference Dose for Aroclor 1016

RISK ASSESSMENT FORUM

REPORT ON THE TECHNICAL REVIEW WORKSHOP ON

THE REFERENCE DOSE FOR AROCLOR 1016

Prepared by:

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Risk Assessment Forum
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Washington, DC

NOTICE

Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Statements are the individual views of each workshop participant; none of the statements in this report represent analyses or positions of the Risk Assessment Forum or the U.S. Environmental Protection Agency (EPA).

This report was prepared by Eastern Research Group, Inc. (ERG), an EPA contractor, as a general record of discussions during the Technical Review Workshop on the Reference Dose for Aroclor 1016. As requested by EPA, this report captures the main points and highlights of discussions held during workshop sessions and includes brief summaries prepared by the workshop chairs of the three technical issues discussed. The report is not a complete record of all details discussed, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear. In particular, each of the three technical issue summaries was prepared at the workshop by the individual chairs based on the panel's discussions during the workshop. Thus, there may be slight differences between the chairs' recommendations.

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FOREWORD

This report includes information and materials from a technical review workshop organized by the U.S. Environmental Protection Agency's (EPA's) Risk Assessment Forum for EPA's Reference Dose/Reference Concentration (RfD/RfC) Work Group. The meeting was held in Washington, DC, at the Barcelo Washington Hotel on May 24-25, 1994. The subject of the technical review was the Integrated Risk Information System (IRIS) RfD entry for Aroclor 1016, a polychlorinated biphenyl (PCB). The expert technical review panel was convened to independently evaluate whether the RfD for Aroclor 1016 is based on a scientifically responsible analysis that represents full consideration of the available data and clear articulation of that analysis in the IRIS RfD entry.

Notice of the workshop was published in the *Federal Register* on May 5, 1994 (59 FR 23202). The notice invited members of the public to attend the workshop as observers and provided logistical information to enable observers to preregister. Observers attending the workshop included representatives from federal government, industry, academia, consulting firms, and the press.

In outlining the scope of the technical review, EPA explained that RfDs are developed using both science and professional judgment. The purpose of this expert technical review is to evaluate and assess the scientific foundation and reasonableness of the IRIS entry. Although a long, and often controversial, history surrounds Aroclor 1016, EPA asked the expert review panel to concentrate on technical issues concerning the selection of a principal study, selection of critical effects, selection of uncertainty factors, and weight-of-evidence conclusions. EPA also requested panel members to consider four broad options for the Aroclor 1016 RfD as potential recommendations to the RfD/RfC Work Group.

A balanced group of 13 expert technical reviewers representing government, academia, environmental groups, and industry were selected to participate in the workshop. Selected reviewers provided scientific expertise in the following disciplines: qualitative and quantitative effects of PCBs in humans and in animals; PCBs and perinatal toxicity; PCBs and neurobehavioral effects; and hazard and risk evaluation for data on health effects other than cancer.

EPA sought comments from these experts on the IRIS entry and related scientific sources. Although EPA would welcome consensus, the expert technical review panel was assembled to generate an array of expert opinions and recommendations. EPA did not expect to resolve all uncertainties in the data and methods associated with the RfD for Aroclor 1016. EPA will use reviewers comments and recommendations and conclusions drawn from this technical review workshop as guidance in considering revisions to the RfD entry and maintaining the scientific integrity of IRIS.

In addition to the technical review experts and observers, the Risk Assessment Forum invited EPA staff who developed the RfD entry to serve as technical resources at the workshop. Also available as technical resources to the workshop participants were Drs. Deborah Barsotti and Susan Schantz, who conducted the underlying studies that served as the basis of the RfD.

The workshop report is organized as follows. The report opens with a brief overview of the workshop and background of the Aroclor 1016 RfD (section 1) and is followed by the chairperson's summary (section 2) and the three chairs' summaries of technical issues discussed at the workshop (section 3). Highlights of the technical reviewers' preliminary comments are provided in section 4. Appendices to the workshop report consist of EPA premeeting materials, including the agenda, list of participants, charge to reviewers, background materials, and premeeting comments (Appendices A-E) and observers and observer materials, including the list of observers and observer comments (Appendices F-G).

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Executive Director and Chair
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SECTION ONE

OVERVIEW

GENERAL SUMMARY

The workshop provided a forum for the expert panel to technically review the scientific underpinnings and reasonableness of all elements of the reference dose (RfD) for Aroclor 1016 as entered into EPA's Integrated Risk Information System (IRIS). IRIS is an on-line database developed by EPA to communicate chronic non-cancer and cancer health hazard information for over 500 substances. Workshop participants contributed useful and substantive suggestions and recommendations for EPA's RfD/Reference Concentration (RfC) Work Group to consider when revising the RfD for Aroclor 1016. Section 3 of this report provides summaries and recommendations prepared by the three workshop chairpersons.

In general, technical reviewers found the principal study to be well conducted. Because the principal study was not designed to evaluate reproductive effects, information that would be useful for assessing the significance of low birth weight as a critical effect could not be easily ascertained. Several technical reviewers discussed whether low birth weight is in fact a sensitive and specific effect. Moreover, some reviewers expressed the opinion that comparisons between exposed groups of test animals might be a more appropriate measure of this effect than comparing test animals to controls. Reviewers also had concerns about the appropriateness of the controls, and the placement of animals from the colony into control groups and test groups was questioned. Technical reviewers all agreed that additional information on weight and the colony was needed to monitor the variability of body weight independent of other effects.

Technical reviewers agreed that the behavioral endpoint of learning and memory deficits should have been addressed in the RfD for Aroclor 1016 and not overlooked due to a lack of expertise within the RfD/RfC Work Group. Reviewers suggested that learning and memory deficits may be of greater concern than low birth weight. Although the studies were considered well

conducted, the research results indicate that further study is warranted to determine the specific deficit or the underlying mechanism for the deficit. Reviewers recommended that EPA obtain adequate expertise to allow full consideration of this "co-critical" effect.

Although some similarities exist between the blue pigmentation effects observed in animals exposed to Aroclor 1016 and Aroclor 1248, discussions indicated that the hyperpigmentation observed in test animals was qualitatively different than the type of pigmentation effects seen in Aroclor 1248-exposed animals. Because limited published data are available on this subject, technical reviewers recommended that the RfD/RfC Work Group consider the reviewers' premeeting comments and workshop discussions in revising the RfD entry. At the time of the workshop, however, reviewers did not consider hyperpigmentation a critical effect for Aroclor 1016.

While discussing exposure issues, reviewers commented that it is difficult to discuss work performed 20 years ago given today's knowledge of polychlorinated biphenyl (PCB) congeners. Reviewers agreed that a body burden existed and that it was dose related, but they could not determine if the measured concentrations were steady state. Reviewers also questioned, without agreement, whether reaching a steady state is an important issue. Nonetheless, reviewers did reach consensus on the recommendation that exposure levels should be recalculated using actual analytically measured concentrations in feed (i.e., 0.700 +/- 0.130 ppm and 0.164 +/- 0.031 ppm) rather than protocol-specified target tissue concentrations (i.e., 1 ppm and 0.25 ppm).

Considerable discussion took place on the issue of whether exposure to chemicals other than Aroclor 1016 occurred. Technical reviewers agreed that the issue of potential contamination by polychlorinated dibenzofurans (PCDFs) required no further action. Several recommendations were made, however, concerning elucidation of whether other PCB contamination, specifically, Aroclor 1248, occurred. These include:

- reevaluating chromatograms (e.g., evaluate confounding factors [peaks 125 and 146]);

- quantitating Aroclor 1248 chromatogram peaks (i.e., look for consistency among similar animals);

- comparing results of infants exposed to either Aroclor 1248 or Aroclor 1016;

- comparing data on infants at birth versus nursing infants;

- examining whether the Aroclor 1016 was contaminated with Aroclor 1248;

- assessing the consistency of non-1016 PCB concentrations across animals;

- determining the source of the contamination; and

- developing a better approximation of the real dose.

In general, technical reviewers had difficulty discussing uncertainty factors issues associated with the RfD for Aroclor 1016. Reviewers pointed out that until the issues associated with critical effects and exposure are resolved, assigning uncertainty factors is premature: Can uncertainty factors be discussed independent of the confidence in the principal study? Technical reviewers suggested that if values other than 10 are chosen as uncertainty factors, then comprehensive justifications should be provided. Moreover, a modifying factor (MF) should be used if the principal study has flaws or uncertainty (e.g., issues concerning the controls or contamination).

Most reviewers expressed the opinion that the primary studies provided sufficient weight of evidence. It was recommended that the results of non-Aroclor 1016 studies should be used in weight-of-evidence conclusions if they are adequately qualified. Given the available data, reviewers were unable to comment on the confidence of the oral RfD for Aroclor 1016 because additional analyses are needed to evaluate the issues identified during the workshop on critical effects and exposure.

A strong difference of opinion was voiced by technical reviewers on which of four options under consideration should be recommended to EPA. The options, as posed in the Charge to Reviewers (see Appendix C), are:

- Option A—Confirm the Aroclor 1016 RfD value with minor refinements.

- Option B—Confirm the Aroclor 1016 RfD value, but revise the text to include an analysis of data limitations and uncertainties.

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■ Option C—Revise the Aroclor 1016 RfD value and accompanying analysis.

■ Option D—Provide other suggestions, including the availability of information published after the RfD was entered on IRIS.

The disparate opinions offered were based on each reviewer's individual level of confidence in the principal studies used to derive the oral RfD for Aroclor 1016.

Among issues discussed, technical reviewers recommended:

■ recalculating exposure levels and the no observed adverse effect level (NOAEL) based on the measured (actual) concentrations in feed; and

■ addressing confidence in the overall RfD by:

— reexamining chromatograms;

— including behavioral effects; and

— evaluating the background (contaminant) levels of exposure to non-Aroclor 1016 chemicals.

BACKGROUND OF REFERENCE DOSE FOR AROCLOR 1016

Aroclors are commercial designations for complex mixtures of PCB isomers and congeners, each consisting of two joined benzene rings and up to ten chlorine atoms. The four-digit number in the Aroclor name indicates the type of isomer mixture (first two digits) and the approximate weight percent of chlorine in the mixture (last two digits). These PCB mixtures can be contaminated with compounds such as PCDFs.

Widespread commercial use of PCBs as dielectrics in transformers and capacitors and as cooling fluids in hydraulic systems, among other uses, began in the 1920s. A general ban of PCBs took effect January 1977 under the Toxic Substances Control Act (TSCA). Although PCBs are no longer produced in the United States, they are stable and persistent chemicals that have been

distributed worldwide. Given the pervasiveness of PCBs, they are currently found in most environmental media, including water, sediments, and soil.

Five years ago, EPA's RfD/RfC Work Group began to develop information for use by scientists and regulators in assessing the risk to humans from exposure to Aroclor 1016 via contact with contaminated environmental media. The work group collected and evaluated a broad range of information in preparing an analysis for the reference dose, focusing on principal studies, endpoint selection, uncertainty factors, and weight- of-evidence analysis. As in all cases, given the range of available information, establishing an RfD is ultimately a product of scientific judgment.

The RfD analysis was completed in December 1992. In January 1993, EPA entered the RfD for Aroclor 1016 into IRIS. Responding to questions raised by an external organization, EPA senior management requested that the IRIS entry be technically reviewed. The Risk Assessment Forum, an EPA group separate from the RfD/RfC Work Group, convened this workshop to gather experts to independently review the RfD for Aroclor 1016 and related scientific sources.

SECTION TWO

CHAIRPERSON'S SUMMARY OF THE WORKSHOP

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INTRODUCTION

On May 24 and 25, 1994, EPA convened a technical workshop in Washington, DC, to assess whether the reference dose for Aroclor 1016 is based on a scientifically responsible analysis that represents full consideration of available data (see Appendix D) and whether that analysis is clearly articulated in the RfD entry on IRIS. Thirteen technical reviewers participated in the workshop (see Appendix A).

In the first part of the Charge to Reviewers (see Appendix C), members of the workshop panel were invited to comment on the major elements of the RfD entry. While comments on all technical aspects of the RfD entry were welcomed, comments on the following four elements were of particular interest:

- selection of the critical study;

- selection of critical effects;

- selection of uncertainty factors; and

- weight of evidence conclusions.

In the second part of the charge, panel members were asked to consider a selection of four possible recommendations concerning the RfD entry. Each reviewer was asked to identify a

preferred option, highlighting primary considerations and noting any suggested changes. The options, listed in full in the Charge to Reviewers, were to:

- confirm the RfD value with minor text refinements;

- confirm the RfD value, but revise the text to include a more comprehensive analysis of the data;

- revise the RfD value and the accompanying analysis; or

- offer other suggestions (e.g., concerning use of data published after the RfD was entered on IRIS in December 1992).

ISSUES

In the workshop panel's deliberations (see section 3 of this report for summaries), issues identified in premeeting comments from review group members were considered under the general topics of critical effects, exposure, and uncertainty factors. An added feature of the meeting was the attendance of the principal investigators who conducted the critical studies. These investigators—Dr. Deborah Barsotti and Dr. Susan Schantz—were on hand to serve as resource persons, and they were called upon frequently to provide additional information relevant to the discussions. Relevant information included confirmation of the lack of randomization or balance in maternal characteristics in assigning animals to treatment groups.

A major barrier to the workshop panel's review was identified as the unavailability of data that had been collected in the critical study. The absence of the information was attributable to the time that had elapsed since the study was completed in 1980 and the objective of the study, which did not concern development of an RfD. Concerning critical effects—one of three specific aspects of the RfD reviewed during the workshop—the group agreed that it was important to obtain the missing information to complete the review. Thus, the group's final recommendations were considered provisional, pending the outcome of an analysis of the missing data. The issues awaiting resolution concerned the appropriateness of the control group and the possible effect that other chemicals besides Aroclor 1016 may have had on the study results.

RECOMMENDATIONS

The workshop panel proposed a two-tiered recommendation. The first part of the recommendation is to revise the NOAEL by using data from the analysis of Aroclor 1016 in chow to calculate administered dose. The NOAEL dose had been calculated from the diet formulation (i.e., the amount of Aroclor 1016 added to the diet). This recommended revision would result in a change in the concentration of Aroclor 1016 in the diet from 0.25 ppm to 0.17 ppm.

The second part of the recommendation is to obtain and analyze chromatogram and maternal weight data from the critical study to provide an understanding of the impact that other chemicals besides Aroclor 1016 had on the observed toxic effects, and to ensure that the control group was appropriate despite the lack of randomization in group assignment. It was the understanding of workshop panel members that these data had been obtained during the critical study, even though the current existence and location of the data were not known. This recommendation represented a strong consensus within the group. Nonetheless, the group was split concerning the degree of confidence that could be placed in the RfD, pending the outcome of additional data analyses.

Eight of the twelve panel members agreed that the statement of "medium" confidence could be made pending the results of the analyses. Two members contended that confidence should be "very low" or "highly uncertain" on the issue of causality pending the results of the analysis. Another member characterized his confidence as "indeterminate" on the issues of background contamination and appropriateness of controls pending the results of additional analyses, and one member said that no confidence statement could be made.

SECTION THREE

REVIEW GROUP SUMMARIES

Critical Effects Issues

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INTRODUCTION

The primary basis for the RfD for Aroclor 1016 is a series of reports on a group of female rhesus monkeys and their offspring published between 1984 and 1991 (see Barsotti and van Miller, 1984; Levin et al., 1988; Schantz et al., 1989; Schantz et al., 1991). The critical effect listed in the RfD was reduced birth weight in rhesus monkey offspring (Barsotti and van Miller, 1984). Postnatal neurobehavioral effects (learning and memory deficits) and transient dermal hyperpigmentation in offspring (Schantz et al., 1989; Levin et al., 1988; Barsotti and van Miller, 1984) also were considered but not listed as critical effects. The purpose of this review group session was to evaluate the scientific basis for:

■ EPA's selection of low birth weight as the critical effect for the Aroclor 1016 RfD;

■ EPA's decision to exclude neurobehavioral effects as a critical effect for the Aroclor 1016 RfD; and

■ EPA's decision to exclude dermal hyperpigmentation as a critical effect for the Aroclor 1016 RfD.

SELECTION OF LOW BIRTH WEIGHT AS THE CRITICAL EFFECT

Background

A significant decrease in the birth weights of rhesus monkey offspring was reported at a maternal dose of 1 ppm Aroclor in diet (0.028 mg/kg-day intake) but not at 0.25 ppm (0.007 mg/kg-day intake) (Barsotti and van Miller, 1984). The study results were used as the basis for the RfD of 0.07 μg/kg-day for Aroclor 1016. Although the mean (+SD) birth weights of rhesus monkey offspring studied were reported (table 1), the report did not include the weights of the individual monkeys, the offspring sex distribution in each group, or information regarding the maternal or paternal characteristics of the groups. The premeeting comments of the review panel indicated that this information is considered crucial for evaluating the scientific basis of the selection of low birth weight as the critical effect for the Aroclor 1016 RfD. Thus, in response to a request for additional information about the study from the review workshop's co-chairs, EPA provided the information shown in tables 2, 3, and 4.

The workshop panel considered the additional information useful, but was concerned about the lack of available data on the parents of the study offspring. Information regarded as particularly relevant included:

- genetic stock of the animals;

- maternal age, weight, parity, time in colony; and

- paternal age, weight, and reproductive history.

Additional information regarding the experimental protocol for control and exposed adults also was considered important.

This information was considered important because these factors also may influence birth weight. Thus, if these factors were not considered in the design of the study, the association between Aroclor exposure and reduced birth weight might not be as straightforward as the one presented in the RfD entry for IRIS. Information regarding possible contamination of the Aroclor diet also was

TABLE 1

WEIGHTS OF INFANTS WHOSE MOTHERS WERE EXPOSED TO AROCLOR 1016 PRIOR TO AND DURING PREGNANCY AND LACTATION

Level of Aroclor 1016 in Diet (μg/g)	Average Birth Weight (g) ($\pm$SD)
1.0 (N=8)	422 (27)
0.25 (N=8)	491 (23)
Controls (N=9)	512 (64)

TABLE 2

ADDITIONAL DATA RELEVANT TO LOW BIRTH WEIGHT: CONTROLS
(Provided by EPA, 5/19/94)

Offspring No.	Sex	Birth Date	Birth Weight (g)	Gestation**	Mother No.	Father No.
AG63	F	4/22/78	510		PP12	PP06
AG65	M	4/26/78	565		PP13	PP02
AG66	F	4/26/78	550		PP14	PP01
AG68	F	5/03/78	400		PP18	1630*
AG70	M	5/04/78	560		PP33	PP05
AG71	M	5/08/78	595		PP37	PP02
AG74	M	5/27/78	495		PP48	1630
AG88	M	8/13/78	570		PP74	PP06
AG96	F	9/28/78	445		PP66	PP02

*Father No. 1630 received Aroclor 1248.
**Data exist but are not available for review.

TABLE 3

ADDITIONAL DATA RELEVANT TO LOW BIRTH WEIGHT: 1-PPM GROUP
(Provided by EPA, 5/19/94)

Offspring No.	Sex	Birth Date	Birth Weight (g)	Gestation	Mother No.	Father No.
AG81	F	7/07/78	430	159	PP79	1632*
AG85	F	7/28/78	410	164	PP88	PP01
AG90	M	8/30/78	480	164	PP89	PP05
AG92	M	9/02/78	385	152	PP70	PP02
AG93	F	9/09/78	440	165	PP87	PP05
AH02	M	10/06/78	405	163	PP76	PP01
AH06	F	10/18/78	425	169	PP81	PP04
AH14	F	12/19/78	405	151	PP64	PP04

*Father No. 1632 received Aroclor 1248.

TABLE 4

ADDITIONAL DATA RELEVANT TO LOW BIRTH WEIGHT: 0.25-PPM GROUP
(Provided by EPA, 5/19/94)

Offspring No.	Sex	Birth Date	Birth Weight (g)	Gestation**	Mother No.	Father No.
AG77	F	5/27/78	480	169	PP83	PP03
AG79	F	7/06/78	495	163	PP82	PP01
AG87	F	8/09/78	470	162	PP56	PP04
AG94	F	9/14/78	515	171	PP75	PP06
AG97	F	9/27/78	495	166	PP62	PP03
AH03	M	10/12/78	450	164	PP67	PP03
AH04	F	10/14/78	525	161	PP72	1632*
AH13	M	12/05/78	500	165	PP78	PP05

*Father No. 1632 received Aroclor 1248.

considered important by the panel; however, since this topic was the focus of a subsequent workshop session, further discussion was postponed.

During the workshop, it was determined that data on the age of the mothers did not exist, since all were feral animals imported to the facility and no effort was made to estimate ages based on dentition. It was also determined that, while the true parity of the mothers was not known, data regarding colony parity were available. Colony parity of the control mothers was thought to be around 3, while colony parity of exposed mothers was known to be 0. The difference was attributable to the length of time that the mothers were in the colony prior to the study; the control mothers were imported in 1973 and the exposed mothers in 1976, approximately 1 year prior to the beginning of the study. While the females were imported 3 years apart, it was determined that the same primate supplier was used for each group and the location of the females prior to importation was likely the same. Although very little data regarding maternal size were included in the original report, maternal weights were noted throughout the study. If available, data on maternal weight at initiation of the study and at critical times during the study would provide important information regarding the possible effects of maternal size or maternal weight gain on birth weight. Weight and reproductive data also were collected on the fathers in the study, but no data exist regarding the age of these animals. In regard to experimental protocol, it was determined at the workshop that all procedures were performed on exposed and control subjects throughout the study.

Review Group Recommendations

While the review group understood the difficulties associated with gathering information on a study that is over 15 years old, it felt that every effort should be made to collect additional data on the parents of the offspring so that the reduced birth weight effect can be adequately evaluated. The work group also made the following specific recommendations:

- Available data regarding characteristics of the parents of offspring studied should be reviewed to provide a better description of the control and exposure groups. Since no data exist regarding parental age and since colony parity and time in the colony are known to differ across the groups, the primary goal of such a review should be to provide further data regarding maternal weight. Data indicating that the maternal weights across the groups were similar would strengthen the conclusion that the

reduced birth weight effect was associated with Aroclor 1016 exposure. Maternal weight gain during the study should also be examined since the little data that were included in the original report indicate that maternal weight gain from the beginning of the study until the end, when the offspring were weaned, was substantially lower for the 1-ppm exposure group when compared to the 0.25-ppm group. This may indicate that the Aroclor exposure hindered normal maternal weight gain during crucial periods of offspring development.

■ The proposed RfD entry indicates a significant reduction in weights for the 1-ppm group when compared to the controls. The results of a statistical comparison of the birth weights of the two exposed groups also should be included in the entry to indicate whether the difference in the birth weights of the exposure groups is significant.

■ The association between Aroclor body burden and birth weight should be investigated. Since data are available regarding PCB levels in skin or fat, analysis of the birth weights according to maternal and offspring body burden using the results of the biopsies should be reported.

■ A comparison of birth weights reported for this study against normative data from rhesus monkeys should be included in the entry. Data from the same colony of animals during the same period of time also would strengthen the basis of the critical effect determination.

EXCLUSION OF NEUROBEHAVIORAL EFFECTS AS A CRITICAL EFFECT

Background

A significant increase in the number of trials required to learn a simple spatial discrimination task was reported for offspring at a maternal dose of 1 ppm Aroclor in diet (0.028 mg/kg-day intake), but not at 0.25 ppm (0.007 mg/kg-day intake) (Schantz et al., 1989). A significant decrease in the number of trials required to learn a simple shape discrimination/reversal task also was reported for offspring at a maternal dose of 1 ppm Aroclor in diet (0.028 mg/kg-day intake), but not at 0.25 ppm (0.007 mg/kg-day intake) (Schantz et al., 1989). Finally, a significant decrease in the percent of correct responses for a delayed spatial alternation task was reported for offspring at a maternal dose of 1 ppm Aroclor in diet (0.028 mg/kg-day intake) when compared to offspring receiving 0.25 ppm (0.007 mg/kg-day intake) but not to controls (Levin et al., 1988).

Schantz et al. (1989) indicated that results of the discrimination-reversal study are consistent with effects attributable to hippocampal damage, as performance on spatial tasks is adversely affected while performance on object-oriented tasks is facilitated. The results of the delayed spatial alternation task, indicating that the 1-ppm offspring performed at a lower rate of correct response than controls while the 0.25-ppm offspring performed at a higher rate than controls, were considered possibly related to the differential effects of exposure on attention (Levin et al., 1988).

The RfD entry indicates that "evaluation of these data is complicated by possible inconsistencies in the outcome of both the discrimination-reversal learning tests (learning was impaired and facilitated on different problems) and the delayed spatial alternation test (performance significantly differed between the two exposed groups, but not between either test group and the control)." Additional information provided to the workshop group indicated that the effects on learning were not chosen as a critical effect due to the "biphasic nature of the response and the lack of statistical power in measuring differences to controls."

Review Group Recommendations

The statements above quoted from the RfD entry do not provide sufficient evidence to discount the inclusion of spatial discrimination effects as a critical effect for Aroclor 1016. Offspring from the 1-ppm maternal dose group required 2.5 times as many trials as the controls to learn the spatial discrimination. The design of the discrimination-reversal study was adequate and the procedures were appropriate for the age of the monkeys. Moreover, the results of the object-oriented discrimination-reversal task should not be considered contrary evidence for an Aroclor effect on spatial learning. The statement regarding "the biphasic nature of the response" addresses concerns regarding the delayed spatial alternation data but does not address the significant learning decrement observed in the spatial discrimination task. As a result, the review group made the following recommendations:

- Discrimination-reversal data should be reviewed to determine whether the number of trials in which the monkeys did not respond (i.e., balk trials) differed across the groups and influenced the results.

■   Data should be provided regarding the performance of the groups across trials (e.g., learning curves).

■   The RfD entry indicates a significant increase in the number of trials that monkeys in the 1-ppm group required to learn a discrimination task when compared to the controls. The results of a statistical comparison of the performance of the two exposed groups also should be included in the entry to indicate whether the difference in the number of trials needed by monkeys in the exposure groups to learn the discrimination task is significant.

■   The association between Aroclor body burden and performance on these tasks should be investigated. Since data are available regarding PCB levels in skin or fat, analysis of the learning data according to maternal and offspring body burden using the results of the biopsies should be reported.

■   Based on the results of the studies at this time, the deficit observed in spatial discrimination learning in the 1-ppm group should be considered a critical effect for Aroclor 1016.

EXCLUSION OF DERMAL HYPERPIGMENTATION AS A CRITICAL EFFECT

Background

Barsotti (1980) reported that "six of the 8 infants of the 1.0 ppm group, 1 of the 8 infants of the 0.25 ppm group and 2 of the 7 infants from the 0.025 ppm group developed hyperpigmentation. These changes were similar to those described in the infants that were exposed to Aroclor 1248." The hyperpigmentation effects were summarized in the RfD entry in the following manner: "Hyperpigmentation was present at birth in the low- and high-dose infants but did not persist once dosing stopped. This clinical change was determined not to be a critical adverse effect."

Review Group Recommendations

No information was provided in the RfD entry concerning the determination not to select hyperpigmentation as a critical effect. Apparently the basis of the decision was that the effect was only transient. After questioning whether hyperpigmentation developed postnatally or was present at birth, the review group determined that the RfD entry was not accurate and that

hyperpigmentation developed postnatally. Details regarding the characteristics of the hyperpigmentation in the exposed offspring were not found in the original report (Barsotti, 1980) or in the RfD entry. Apparently the hyperpigmentation was not similar to the chloracne and pigmentation observed in humans exposed to tetrachlorodibenzo-p-dioxin (TCDD); rather, it appeared as an exaggeration of the blue pigmentation pattern commonly seen in rhesus neonates. Based primarily on the fact that the hyperpigmentation does not match clinical signs in humans, the review group recommended the following:

- The RfD entry should be corrected to indicate that the hyperpigmentation was not present at birth but developed postnatally.

- The hyperpigmentation should be described in detail and compared to the known clinical signs in humans of PCB exposure, such as chloracne and pigmentation.

- Based on the results of the study, at this time hyperpigmentation should not be considered a critical effect for Aroclor 1016.

Exposure Issues

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INTRODUCTION

Premeeting comments on exposure issues that were addressed by the group focused on two minor areas and one major aspect of the critical study. The two minor issues discussed concern the doses of Aroclor 1016 that the rhesus monkeys in the study received and whether steady-state conditions were reached before the animals were bred. The major issue discussed concerns whether the study animals were exposed to other chemicals, and if so, what the levels of those other exposures are and what the effect of these exposures might be on the outcome of the study and on the proposed RfD for Aroclor 1016.

DOSE

Background

The monkeys were fed diets calculated to contain 1.0 ppm, 0.25 ppm, and 0 ppm of Aroclor 1016. These concentrations were used to calculate the average total intake of 18.41 +/- 3.64 mg/kg and 4.52 +/- 0.56 mg/kg for the 1.0-ppm and 0.25-ppm dose groups, respectively (Schantz et al., 1989). The three diets were analyzed for Aroclor 1016 content and the actual concentrations were 0.700 +/- 0.130 (N=12) ppm, 0.164 +/- 0.031 (N=12) ppm, and 0.005 +/- 0.001 (N=9) ppm in the 1.0-ppm, 0.25-ppm, and control chow groups, respectively.

Review Group Recommendations

The review group recommended that dose levels be recalculated using the actual concentrations, which would result in a reduction in dose levels of about 30 percent. The group also recommended that an attempt be made to correlate body burden measurements with health endpoint data.

STEADY-STATE CONDITIONS

Background

The issue of whether the monkeys' body burden had reached steady-state conditions at the time of conception could be important for determining the uncertainty factor if the study is determined not to be a chronic study. In the study, the level of PCBs in the adipose tissue increased during the dosing period between 4 months and 7 months, which could indicate that steady-state conditions had not been reached. To reach a conclusion on this issue, however, a pair-wise comparison would have to be made rather than using a comparison of the average Aroclor 1016 adipose tissue concentrations.

Review Group Recommendations

The review group considered the data inadequate to reach a conclusion on steady-state conditions and felt that the issue is unlikely to be resolved. Thus, the review group found no basis for recommending that the issue be investigated further.

POSSIBLE EXPOSURE TO OTHER CHEMICALS

Background and Discussion

The review group focused much of its discussion on the possibility that the monkeys were exposed to other substances besides Aroclor 1016. Along with exposure to Aroclor 1016 in the control diet, specific substances that were discussed include PCDFs and other possible Aroclors, particularly Aroclor 1248.

Barsotti and van Miller (1984), Barsotti's thesis (1980), and the IRIS document all stated in slightly different ways that the Aroclor 1016 fed to the monkeys did not contain chlorinated dibenzofurans (CDFs). This issue was clarified by Dr. Deborah Barsotti, who stated at the workshop that J. McKinney had analyzed the Aroclor 1016 used in the experiments to determine the tri-, tetra-, penta-, and hexa-CDF content. None was observed at the detection limit of 5 ppb. The consensus within the review group was that this additional information clarified the issue and that no further information was needed.

Another exposure issue concerned whether Aroclor 1016 was in the diet of the control animals. This issue was raised because Barsotti (1980 at p. 185) states, "Control adipose samples contained PCBs based on Aroclor 1016 standard at the level of 0.69 +/- 0.38 $\mu g/gm$ on the lipid basis. Three animals sampled had adipose PCB levels below the limit of confident detection for this analysis." This finding is unexpected because there was no known source of exposure to Aroclor 1016. Dr. Barsotti stated that the analytical results were quantitated as Aroclor 1016, without a specific determination as to which Aroclors were present. The PCB mixture might have come from the fish meal in the Purina monkey chow. The Great Lakes fish used in the chow contained PCBs, although probably not Aroclor 1016. Dr. Barsotti's statement helped to alleviate the concern and clarified that Aroclor 1016 was not known to be in the control diet. This issue could be clarified, however, by looking at the chromatograms and confirming the identity of the PCB mixture.

Most of the review group's discussions about exposure focused on the likelihood of exposure to other possible Aroclors, particularly Aroclor 1248. Barsotti (1980 at p. 192) states, "We have shown that the concentration of PCBs in control monkey chow is in the range of 1 - 50 ppb on the

basis of an Aroclor 1248 standard." A second question about Aroclor 1248 exposure arose because peaks with relative retention times of 125 and 146 were on the gas chromatograms of the tissue samples; these peaks may be indicative of Aroclor 1248 exposure.

The importance of possible Aroclor 1248 exposure depends on whether contamination is consistent across all exposure groups. This is partially dependent on whether the contamination was in the chow from the manufacturer or whether some contamination was introduced during the pelleting operation. Judging from premeeting comments, some review group members considered this a relatively minor issue because (1) the contamination appeared to be consistent across all dose groups and at orders of magnitude less than the Aroclor 1016 exposure, (2) body burden data were available, and (3) hyperpigmentation was not observed in the control animals. Others considered this a major issue, given that some of the effects can be caused by Aroclor 1248 (or other, dioxin-like chemicals), and a causal association cannot be established without a definitive chemical characterization of the diet.

Review Group Recommendations

The review group made the following recommendations:

- The 1.0-ppm, 0.25-ppm, and control chow were analyzed 12, 12, and 9 times, respectively, throughout the study. Data and chromatograms should be examined to determine the Aroclor 1248 content.

- Chromatograms for the tissue analyses could be examined, looking for consistency among similar animals in levels of peaks that are not from Aroclor 1016. The data for the control and exposed animals would have to be reviewed separately because the control animals were older and had been on commercial chow longer and, thus, would be expected to have higher levels of Aroclor 1248 in their tissues.

- Chromatograms for the tissues of the offspring could be reviewed. Reviewing results for tissues at birth might be easier and would have less confounding information than the chromatograms of tissues after nursing. Levels of individual peaks from tissues after nursing would be more complex because of greater differences in, for example, metabolism and excretion when the chemicals were ingested with milk in contrast to exposure *in utero*.

- Chromatograms from tissues of the Aroclor 1016 infants could be compared to those from the Aroclor 1248 infants, looking for similarities. This information could help to determine the magnitude of the exposure to Aroclor 1248.

- Birth weight study results from the infants on Aroclor 1248 could be compared with the birth weight results for the infants on Aroclor 1016. For example, the average birth weight in the 1.0-ppm Aroclor 1016 infants was 422 g +/- 29 g and the average birth weight of the Aroclor 1248 infants was 476 g +/- 42 g. The experimental design would have to be compared; however, these data, at first glance, suggest that Aroclor 1016 has a greater effect on birth weight than Aroclor 1248. If this were true, contamination by Aroclor 1248 may be of less importance.

- In reviewing the chromatograms, one would need to keep in mind the difference in the pharmacokinetics of the various congeners.

- The chromatogram of the Aroclor 1016 mixture that was used to prepare the chow could be examined to look for Aroclor 1248 peaks.

- In general, when reviewing the chromatograms, two different approaches are possible. One approach would be to "eye-ball" the similarities or differences; the other would be to quantitate the different peaks. It may be possible to combine these approaches in an efficient manner. For example, "eye-balling" a number of chromatograms may help to suggest an answer to some of these questions. Then the peaks of a minimal number of chromatograms could be quantified to document the findings.

Uncertainty Factors Issues

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INTRODUCTION

The review group's consideration of uncertainty factors (UFs) began with a review of changes that EPA's RfD/RfD Work Group made during development of the RfD between January 1990 and January 1993, when the entry was loaded onto IRIS. During this development process, a number of alternatives were considered for the value of the five UFs. In addition, the statement concerning weight of evidence, the studies cited as supporting the RfD, and the statement and justification for the section on confidence evolved in response to discussions by the RfD/RfC Working Group and comments received from an EPA internal peer review.

ISSUES IDENTIFICATION

The workshop's technical review group began its discussions with identification of several issues concerning UFs derived from the workshop's premeeting comments:

- Some review group members felt that the value of the composite uncertainty factor was appropriate and were not concerned about the distribution among the various component UFs. One reviewer suggested eliminating the presentation of individual UFs.

- The UF_S (subchronic to chronic) received the most attention in premeeting comments. All possible alternatives for the UF_S were suggested by individual reviewers, including not using this factor and using values of 1, 3, or 10. Use of these options would depend on whether the critical study was considered to be subchronic, chronic, or "more than subchronic, less than chronic."

- Values of 1 rather than 3 were suggested for the UF_A (interspecies extrapolation) and the UF_H (intraspecies extrapolation) based on the strong similarity of non-human primates to humans and the strong identification of the fetus as a member of a sensitive population.

- Two reviewers commented on the possibility of reconsidering the dose selected as the NOAEL and altering UFs based on this result. One review group member suggested that the 0.25-ppm diet group be used as the LOAEL rather than the NOAEL. Another reviewer suggested that a modifying factor (MF) be added to take into account that the NOAEL might be lower if a benchmark dose, based on modeling of the dose-response curve, were used.

REVIEW GROUP RECOMMENDATIONS

Weight of Evidence

The review group considered it important to discuss the weight of evidence section of the RfD entry before addressing issues concerning UFs. The discussion that ensued focused on whether studies that did not use Aroclor 1016 dosing should be cited as supporting evidence. This group of studies primarily report on the consequences of human exposure to PCBs via environmental media.

The review group recommended that these studies not be used as supporting evidence for hyperpigmentation since the effect in monkeys did not resemble that reported for TCDD/PCB exposure in humans. In general, studies in humans where exposures were not limited to Aroclor 1016 should not be cited as supporting evidence, the review group recommended, unless the effects reported were similar to those observed in the critical study for the RfD. Even then, the degree of support provided by such studies should be qualified. This recommendation was made specifically in regard to information from the Yusho and Yu-Cheng poisoning incidents (Rogan, 1989), and the studies of birth weight from Michigan populations exposed to PCBs via fish consumption.

Similarly, the review group suggested that more emphasis be given to the studies of Taylor et al. (1984, 1989), in which occupational exposures were more specific to Aroclor 1016. A review group member also commented that Aroclor 1016 had a fairly unique or characteristic composition of congeners compared to other PCB commercial mixtures. Thus, scientific data comparing mixtures suggest that data from environmental exposures in humans is not as valuable as supporting evidence for Aroclor 1016 as for some other commercial mixtures.

On another issue, one review group member said that a discussion of the differences between mink and human reproductive characteristics should be included if data on reproduction in mink are cited as supportive. Another group member suggested that the section on supporting studies be reorganized and reworded to reflect the relative weight of supporting studies.

Values for Uncertainty Factors

In the discussion about values for specific UFs, review group members noted that, while non-human primates are known to be similar to humans, there are major differences between non-human primate species. Moreover, the rhesus monkey is not necessarily representative of all non-human primates. A group member pointed out that the supporting studies on similarity between monkeys and humans in PCB metabolism and clearance were limited to two congeners. Thus the UF_A was appropriately set at 3 rather than 1. Review group members also commented that use of data developed by Hugh Tilson (of the EPA Health Effects Research Laboratory at Research Triangle Park, NC) to justify behavioral outcome similarities between monkeys and humans should be qualified by the fact that these studies did not use Aroclor 1016.

A review group member commented on the UF_H, noting that if behavioral endpoints were considered co-critical, the RfD's statement on transplacental exposure would not be appropriate since transmammary exposure also occurred. A fairly extensive discussion of the history and value of the use of behavioral endpoints for RfD determination followed. A consideration of problems and issues past and present resulted in general agreement regarding qualified support for the use of behavioral endpoints in RfD development.

The review group did not feel it appropriate to discuss the UF_S and UF_D (database deficiencies). One review group member, however, provided a suggestion and strong justification for use of an MF to cover issues of background contamination and appropriateness of controls. When carried forward to other discussions, this suggestion retained its strength.

Confidence

Discussion of the RfD entry's section on confidence immediately focused on the importance of the analysis of chromatograms and weight data in allowing selection of a level of confidence. This discussion carried over into formulation of a recommendation from the workshop panel in the final session of the meeting.

SECTION FOUR

HIGHLIGHTS FROM TECHNICAL REVIEWERS' PRELIMINARY COMMENTS

Prior to the workshop, each technical reviewer was asked to prepare written comments on the major elements of the RfD entry, covering selection of a principal study, selection of critical effects, selection of uncertainty factors, and weight-of-evidence conclusions. Relying on their technical knowledge and best professional judgment, reviewers also considered four broad options as potential recommendations to the RfD/RfC Work Group. Appendices C and D provide the Charge to Reviewers and background information, respectively. Technical reviewers' premeeting comments are presented in Appendix E.

Three critical health effects issues were identified by technical reviewers:

- low birth weights;

- learning and memory deficits; and

- hyperpigmentation.

The point was made by several reviewers that the principal study was not designed as a reproductive study; rather, it was designed to investigate metabolic issues—the low birth weight effect was unexpected. As a result, reviewers identified the following factors other than Aroclor 1016 as important for evaluating the low birth weight effect:

- maternal age;

- maternal weight and weight gain;

- maternal parity;

- time in colony;

- paternal factors (e.g., age, weight, time in colony, and reproductive history);

- colony versus study controls (i.e., What was the protocol during the study?);

- contamination of diet;

- Aroclor 1016 feeding protocol;

- paternal exposure;

- external factors (e.g., housing and temperature);

- identification of genetic stock of controls and test animals;

- randomization; and

- comparison of exposed animals only.

Regarding the last two factors, many comments were made on the sources of animals and whether any counterbalancing occurred in selecting test and control animals from different groups. Reviewers inquired, for instance: How representative were control animals to test animals? Were they totally randomized? If different exposures are being compared, reviewers suggested, then randomization is a critical factor that must be considered.

To measure learning and memory, test animals were tested at 14 months and 4 years of age using several discrimination-reversal (DR) problems (e.g., simple spatial DR, modified spatial DR, color DR, shape DR, and delayed spatial alternation). Technical reviewers questioned why learning and memory deficits were not listed as a "co-critical" effect. Reviewers also took issue with the reason this effect was not chosen as critical: the reasoning was based on the biphasic nature of effects and the lack of statistical significance from controls, but reviewers pointed out that DR is considered a straight effect, not biphasic. Technical reviewers also observed that deficits in original learning and borderline effects on reversal are similar to deficits observed in non-human primates with discrete lesions on the prefrontal cortex. Other data identified as necessary for assessing this effect are general study conditions, postnatal exposures, and learning curves.

As with behavioral endpoints, reviewers questioned why hyperpigmentation was not considered a "co-critical" effect. Comments covered such issues as whether hyperpigmentation is a clinical or a toxicological effect. Reviewers also queried: Is the hyperpigmentation an indication of exposure and therefore an effect? If the finding is similar and consistent regarding effects with

other chlorinated compounds, can it be ignored? It was noted that hyperpigmentation is usually associated with exposures to PCBs with higher chlorination than Aroclor 1016.

Technical reviewers' comments addressed three issues related to exposure:

■ Was a steady-state concentration reached?

■ Was the exposure dose calculated using the protocol-specified target tissue concentration (i.e., 1 ppm) or the actual concentration measured in the feed (i.e., 0.7 ppm)?

■ What other chemicals were measured (e.g., PCDFs, Aroclor 1016 in control diets, Aroclor 1248, other PCBs) and what concentrations were detected?

Referring to Table 6-1 in Dr. Deborah Barsotti's thesis (1980) on levels of PCBs in the adipose tissue of female monkeys consuming Aroclor 1016, comments were made on the different levels observed between the 1-ppm and 0.25-ppm groups. The variance observed in the 1-ppm group was explained by the rapid metabolism and excretion of most Aroclor 1016 congeners. Because only total PCBs were measured, however, it is difficult to tell which congeners were at a steady state.

Because samples were collected and analyzed for every batch of feed prepared, the concentrations measured were considered representative of the entire study period. Exposure was calculated, however, using the protocol-specified target tissue concentrations of 1 ppm (18.41 mg/kg) and 0.25 ppm (4.52 mg/kg), rather than the actual measured levels (0.700 +/- 0.130 and 0.164 +/- 0.031) in the feed. Reviewers indicated that doses should be recalculated based on actual measured concentrations in the feed.

Many comments addressed the potential contamination of the feed with other chemicals. The Aroclor 1016 stock was analyzed for PCDFs, but measured concentrations were less than 5 ppb, the analytical detection limit. Concern was raised over the presence of Aroclor 1016 in five out of eight animal's adipose tissue before dosing occurred; Aroclor 1016 would not be expected. It was pointed out, however, that the commercial monkey chow contained fish meal that was prepared from fish caught in the Great Lakes (which is contaminated with PCBs). The monkey chow manufacturer certified that a minimum amount of PCBs was present, but would not specify which congeners.

Low levels (0.50 ppb) of Aroclor 1248 and other PCBs were detected in the commercial monkey chow, and conclusions about the presence of these non-Aroclor 1016 PCBs were made based on observed effects (i.e., hyperpigmentation). The lack of information on the processing and analysis of feed for control and test animals, however, left many questions unanswered:

- Was the food contamination from the manufacturer or from the pelleting operation?

- What analytical data on tissues and diet are available?

- When was the feed analyzed?

- Was the feed processed differently for control and test animals?

- If Aroclor 1248 was present in the feed, why were effects not seen in controls and at all exposure levels?

- What effect does inter-animal variability have on the results?

Various explanations for the presence of Aroclor 1248 were presented, including:

- Since Aroclor 1248 also was being studied at this facility at the same time studies were being conducted on Aroclor 1016, some of the animals could have inadvertently been exposed to Aroclor 1248.

- Aroclor 1248-like peaks on chromatograms could be the result of bioaccumulation, given the nature of the congeners.

One reviewer asked: At what level of contamination does the Aroclor 1016 data become unusable?

Although technical reviewers generally considered the total uncertainty factor of 100 selected for the Aroclor 1016 RfD to be reasonable for the chosen critical effect, several reviewers raised issues concerning the assignment of individual uncertainty factors:

- If little concern exists about the distribution and justification of individual uncertainty factors (e.g., UF_A, UF_H, UF_D, and UF_S), could the discussion of individual uncertainty factors be eliminated?

- Uncertainty factors for interspecies (UF_A) and intraspecies (UF_H) extrapolations should not both rely on species similarities. Also, species similarities should not be used unless sufficient data exists to support such a determination.

- The uncertainty factor for extrapolation from subchronic to chronic exposures (UF_S) should be reevaluated based on a determination of whether (and when) a steady-state body burden had been reached.

Technical reviewers' comments on the weight-of-evidence conclusions spanned from the specific to the general. Overall, many of the reviewers found that the primary studies provided sufficient weight of evidence. One reviewer suggested that the discussion should be reorganized to reflect the two-fold purpose of the section: to summarize the remaining literature and to justify the critical study. Several reviewers recommended that the mink studies not be used as supporting evidence. Another reviewer asked that the role of behavioral studies as supporting evidence be clarified.

Reviewers' preliminary comments indicated that all four recommendations posed in the Charge to Reviewers—options A, B, C, and D—were considered viable recommendations, with more reviewers favoring options B and C. If, however, the exposure levels are adjusted for the actual concentrations in the feed versus what was called for in the protocol, then options A and B can be eliminated since the NOAEL will be recalculated. One reviewer suggested that the data be reexamined to determine if a basis exists to support the RfD.

SECTION FIVE

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APPENDICES A-E

EPA PREMEETING MATERIALS

APPENDIX A

FINAL PARTICIPANT LIST

U.S. Environmental Protection Agency

TECHNICAL REVIEW WORKSHOP ON THE REFERENCE DOSE (RfD) FOR AROCLOR 1016

Barcelo Washington Hotel
Washington, DC
May 24-25, 1994

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APPENDIX B

AGENDA

U.S. Environmental Protection Agency

TECHNICAL REVIEW WORKSHOP ON THE REFERENCE DOSE (RfD) FOR AROCLOR 1016

Barcelo Washington Hotel
Washington, DC
May 24-25, 1994

AGENDA

Co-Chairs

Dr. Mari Golub
California Environmental Protection Agency
University of California - Davis

Dr. Thomas Burbacher
School of Public Health and
Community Medicine
University of Washington

Dr. Nancy Kim
Division of Environmental Health Assessment
New York State Department of Health

TUESDAY, MAY 24

7:30AM	Registration and Onsite Check-In
8:30AM	**Welcome** Dr. Dorothy Patton Risk Assessment Forum U.S. Environmental Protection Agency **Introduction/Workshop Structure** Dr. Golub **Effects Issues** **Chair: Dr. Burbacher**
8:45AM	Summary of Comments
9:00AM	Panel Discussion
10:15AM	**BREAK**
10:30AM	Panel Discussion (continued)
12:15PM	Wrap Up

TUESDAY, MAY 24 (continued)

12:30PM	**LUNCH**

Exposure Issues
Chair: Dr. Kim

1:45PM	Summary of Comments
2:00PM	Panel Discussion
3:30PM	Wrap Up
3:45PM	**BREAK**
4:00PM	**Observer Comments**
5:00PM	**Adjourn**

WEDNESDAY, MAY 25

Uncertainty Factor Issues
Chair: Dr. Golub

8:00AM	Summary of Comments
8:15AM	Panel Discussion
10:00AM	Wrap Up
10:15AM	**BREAK**
10:30AM	**Workshop Panel Recommendations to EPA** Dr. Golub
12:00NOON	**Adjourn**

APPENDIX C

CHARGE TO REVIEWERS

CHARGE TO REVIEWERS
FOR THE RfD FOR AROCLOR 1016

As described in the background section, EPA's RfD/RfC work group collects and evaluates a broad range of information in preparing an analysis for each reference dose, focussing on principal studies, endpoint selection, uncertainty factors, and weight of evidence analysis. In all cases, a range of information exists and the RfD is ultimately a product of scientific judgment. The issue for the peer review is whether the RfD for Aroclor 1016 is based on a scientifically responsible analysis that represents full consideration of the available data and clear articulation of that analysis in the RfD entry on IRIS.

This charge has two parts. The first part invites highly specific comments on the major elements of the RfD entry (Attachment 1). The second part asks peer reviewers to consider four broad options as potential recommendations to the RfD work group.

Note: The RfD analysis was completed in December 1992. For this reason, references and analysis do not include research published after that date. See Part II, Option D.

NOTE: For your information, the RfD/RfC work group and the Risk Assessment Forum are separate agency organizations. When questions were raised about the RfD entry for Aroclor 1016, senior agency management asked the Risk Assessment Forum staff to organize an independent technical review. The Forum will collect reviewer comments and workshop recommendations and conclusions, and make these materials available to the RfD/RfC work group, senior agency management, and the public. This charge was prepared by the RAF staff based on materials prepared or supplied by RfD/RfC work group members.

Part I. Comments. While EPA invites comment on any and all technical aspects of the RfD entry for Aroclor 1016, we are particularly interested in comments and analysis on the four major elements in the RfD for Aroclor 1016.

Selection of Principal Study

Typically, EPA's RfD/RfC work group bases an RfD on a single experimental value, the NOAEL or the LOAEL, derived from a single "principal" toxicity study. The study designated as the principal study will usually have the lowest NOAEL and/or lowest LOAEL among all the studies evaluated. In addition, the principal study must be of sufficient quality, clearly identify effects observed at the NOAEL and LOAEL, and must be supported by the weight of evidence of the entire data base.

1. The principal study used for the Aroclor 1016 RfD was
    published as four periodic reports on a single group of
    rhesus monkey mothers and their offspring, including
    follow-up data for up to four years after birth. The
    four reports (Barsotti and van Millar, 1984; Levin et
    al. 1988; Schantz et al; 1989; Schantz et al; 1991),
    were all drawn from Dr. Barsotti's doctoral
    dissertation and each was published in the peer review
    literature.

2. The principal study has not been corroborated in a
    second non-human primate (NHP) species. However, the
    RfD work group concluded that evidence of adverse
    health effects observed in the rhesus monkeys in this
    study was consistent with data from certain studies in
    other species including macaque monkeys, pastel mink,
    rats, and humans.

3. The authors of the principal study, as well as various
    independent analyses, have identified several factors
    that have led to questions about the principal study.
    These factors include: low level Aroclor 1048
    contamination of the diet; small and variable treatment
    group sizes; control animals held under laboratory
    conditions for longer time periods than the treated
    animals; exclusion of the lowest dose group from
    published reports because of PBB contamination of the
    feed; questions about conformity with GLP standards.

4. The RfD workgroup evaluated the principal study in
    terms of each of these factors, including additional
    data analysis, and concluded that these factors did not
    disqualify use of these reports as the principal study.
    These considerations are described in the RfD meeting
    notes.

Please review Attachment 1 and comment on selection and use of
the four reports listed above as the primary basis for the RfD
for Aroclor 1016, including the relevance of other studies,
questions raised about the principal study, and any other
considerations bearing on the scientific reliability of this
study for this purpose.

Selection of Critical Effects

Observations in the rhesus monkeys used in the principal study and in other species demonstrate that pre-natal exposure to PCBs may affect many organ systems.

1. Reduced birth weight in the rhesus monkeys in the principal study was identified as the critical effect for the RfD, and postnatal neurobehavioral effects and transient dermal pigmentation attributed to Aroclor 1016 exposure were also considered.

2. Endpoints observed in other studies included chloracne, dermal pigmentation, facial edema and biochemical changes in the central nervous system in monkeys; adrenal effects in rats, immunologic effects in mice, and reproductive effects in mink. Although this information was regarded as consistent evidence of PCB effects, none of these effects was used as the basis for the RfD.

3. The RfD workgroup considered the confounding factors listed above (see page 2) and concluded that these factors did not compromise the data on reduced birth weight in these animals.

4. Certain human studies show comparable effects in the offspring of women exposed to PCB mixtures occupationally or from eating PCB-contaminated fish. This is consistent qualitatively with the animal studies on the general question of PCB exposure, but is not specific for Aroclor 1016.

Please review Attachment 1 and comment on selection of low birth weight as the critical effect for the Aroclor 1016 RfD, along with information on postnatal neurobehavioral effects.

Selection of Uncertainty Factors

When the Agency develops an RfD, it first considers whether there is a minimum data base available. If only a minimum data base (a single well conducted subchronic study that only defines a LOAEL) is available, the Agency considers five area of uncertainty and quantitatively accounts for them. These areas of uncertainty include interspecies extrapolation (UF_A), intraspecies extrapolation (UF_H), database deficiencies (UF_D), subchronic to chronic exposure extrapolation (UF_S), and LOAEL to NOAEL extrapolation (UF_L). An explanation of how the Work Group quantitatively accounted for uncertainty in the Aroclor 1016 database is included below.

1. The RfD workgroup practice is to evaluate five full areas of uncertainty that are assigned a factor of 10 unless other data suggest a factor less than 10 (usually 1 or 3).

2. For Aroclor 1016, the RfD/RfC Work Group used four areas of uncertainty for interspecies extrapolation (UF_A), intraspecies extrapolation (UF_H), database deficiencies (UF_D), and subchronic to chronic exposure extrapolation (UF_S). For each UF application the available information warranted the use of a factor of less than 10.

    * UF_A = 3 - Similarity of NHP to human in general, and specifically metabolism of PCBs and response to PCB (chloracne, developmental toxicity, neurobehavioral effects) warrant use of half-log UF.

    * UF_H = 3 - Transplacental exposure of infants to PCB indicate sensitive subpopulation. Appropriate use of NHP as principal study argue for less than 10.

    * UF_D = 3 - An extensive database for both human exposure and animal studies is available. However, since specific studies relating to male reproductive effects and two-generation reproductive studies were not available, a half-log factor was used. Also, it is expected that PCBs will cause the same reproductive effects in humans.

    * UF_S = 3 - A true chronic exposure study was not conducted in monkeys, although the duration of exposure was considered much greater than subchronic and long-term latent effects were observed. The mothers were probably dosed to "steady state" and were exposed for all of gestation. It is apparent from the human data that the developmental effects are most likely the most sensitive critical effect and the availability of a chronic duration study would not necessarily provide a more sensitive NOAEL. Therefore, a total of less than 10 is warranted.

3. Total UF = 100 is lower than average when compared to other RfD files examined by the Work Group. This addresses the overall strength of the file, confidence in the file and assuredness in identification of a dose-response for the most sensitive endpoint in a most relevant species, rhesus monkeys. The RfD work group

concluded that the identified deficiencies of the principal study are offset by the weight of evidence of the supporting data.

Please review Attachment 1 and comment on the uncertainty factor analysis for Aroclor 1016.

Weight of Evidence Conclusions

The RfD for Aroclor 1016 is based on evaluation of several lines of animal and human evidence.

Primary Evidence Used for the RfD

1. The NHP study provides conclusive data that the reduced birth weight of infants and neurobehavioral effects is consistent with effects observed in other species including the human. Birth weight has also been affected in mink; behavioral effects have been noted in rodents.

2. The developmental and neurobehavioral effects in the NHP are considered most predictive of effects in humans, although methodological considerations precluded using the neurobehavioral effects and transient dermal pigmentation as co-critical.

Secondary Evidence

1. In another NHP study with Aroclor 1016, investigators have reported a change in dopamine concentration in different brain regions.

2. Chloracne and pigmentation have been observed in both NHP and humans.

3. Mink have also been demonstrated to have an adverse developmental effect (decreased fetal birth weight and reduced litter size) following Aroclor 1016 exposure.

4. *In vitro* metabolism of several PCB congeners is similar for NHP and humans.

Evidence less consistent with the RfD conclusions

1. There is difficulty in assessing human response-exposure to a mixture of congeners. Additionally, the chlorination profile is difficult to characterize both in human exposures and in environmentally occurring mixtures.

2. The developmental toxicity and neurobehavioral effects are not well characterized in rodent species. However, the NHP is judged to be a better predictor of human effects as demonstrated by the quantitative data of the principal study and the qualitative observations of humans. The behavioral effects were not chosen as critical given the biphasic nature of the response and the lack of statistical power in measuring differences to controls.

Please review Attachment 1 and comment on the weight of evidence analysis.

Part II. Recommendations. A range of recommendations might arise from the peer review. We are interested in your recommendation as to four broad options.

1. **Option A.** Confirm the Aroclor 1016 RfD value as presented in the IRIS entry, with minor refinements in the text as recommended during this peer review.

2. **Option B.** Confirm the Aroclor 1016 RfD value as presented in the IRIS entry, but revise the text to include a more comprehensive analysis of data limitations and related uncertainties.

3. **Option C.** Revise the Aroclor 1016 RfD value and accompanying analysis in line with peer review recommendations.

4. **Option D.** Any other suggestions, including suggestions regarding information published after the RfD was entered on IRIS in December 1992.

Please identify your preferred option, highlighting the primary considerations influencing your choice, along with any recommended changes.

APPENDIX D

BACKGROUND INFORMATION FOR TECHNICAL REVIEWERS

Background Information

Reference Dose/Reference Concentration (RfD/RfC). An RfD or RfC is based on the assumption that thresholds exist for certain toxic effects, e.g., cellular necrosis, but may not exist for other toxic effects e.g., carcinogenicity. Generally, an RfD or RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Accompanying the RfD or RfC are statements on uncertainty and EPA's confidence in the RfD or RfC and in the underlying data.

RfD/RfC Work Group. The purpose of the RfD/RfC Work Group is to reach consensus on oral RfDs or inhalation RfCs for noncancer chronic human health effects developed by or in support of program offices and the regions. The work group also works to resolve inconsistent RfDs/RfCs among program offices and to identify, discuss, and resolve generic issues associated with methods used to estimate RfDs/RfCs.

Scientists from a mix of pertinent disciplines are selected by executive appointment from all major Agency program and regional offices. In addition, scientists from ATSDR and FDA are invited to work group meetings as observers to assist the Agency in the information gathering process.

The RfD/RfC Work Group usually meet every other month for two days. Substances are discussed at the request of any Agency office or region. The requesting office generally prepares a file that consists of a summary sheet, a copy of the critical study, and supporting documentation.

The work group meets to review the file and determines if the substance-specific summary is adequate. Work group consensus <u>must</u> be reached on each RfD or RfC. (Note: Consensus generally means that no member office is aware either of information that would conflict with the RfD or RfC, or of analyses that would suggest a different value that is more credible.) Once unanimous consensus is reached, the substance-specific summary for the RfD or RfC is prepared for inclusion on IRIS.

In some cases, the work group agrees that adequate information is not available to derive an RfD or RfC. A message is then put on IRIS, which states that the work group reviewed the specific substance and determined that the health effects data for the substance were inadequate for derivation of an RfD or RfC.

Scope of the Technical Review. Although any discussion of EPA's RfD program presents several generic issues, the May technical review meeting is sharply focused on the science-based

information and analyses relevant to Aroclor 1016. Several limitations are important.

First, the technical review of the Aroclor 1016 RfD entry focuses on scientific and technical issues for that chemical only. Larger issues regarding the generic RfD process are being addressed by the Agency as a separate effort (58 FR 11490; February 25, 1993).

Second, while we would welcome consensus, the issues are complex and EPA does not expect the technical reviewers to reach consensus on all issues raised in the workshop. Rather, the purpose of this workshop is to collect expert opinions and recommendations on reasonable approaches to some difficult and potentially controversial scientific issues.

Finally, EPA does not expect to resolve all uncertainties in the data and methods associated with the RfD for Aroclor 1016. However, with the help of the technical reviewers, EPA expects to identify the most important areas of uncertainty, useful options for addressing the uncertainties, and the expected impacts of these uncertainties on the RfD entry.

IRIS. The Integrated Risk Information System (IRIS) data base, developed by the U.S. EPA, contains Agency consensus positions on the potential adverse human health effects of approximately 500 specific substances. IRIS is the Agency's primary vehicle for communication of this information following comprehensive review by intra-Agency work groups. EPA developed IRIS for Agency staff in response to a growing need for consistent risk information on chemical substances for use in decision-making and regulatory activities.

NOTE: For further information, refer to the attached IRIS Fact Sheet.

RfD File. A public file is maintained on each IRIS entry and is available for inspection in Cincinnati. Documents contained in the file may include but are not limited to the RfD entry, the principal study as identified in the IRIS summary sheet and other supporting toxicological studies, and final work group meeting notes.

⊕EPA **IRIS** Integrated
Risk
Information
System

The Integrated Risk Information System (IRIS) data base, developed by the U.S. Environmental Protection Agency (EPA), is EPA's primary vehicle for communication of chronic non-cancer and cancer health hazard information for over 500 substances. This information is summary in nature, representing Agency consensus positions following comprehensive review by intra-Agency work groups. IRIS is a resource that points the user to the underlying human and/or animal data used to support the Agency's position.

The system contains hazard identification and dose-response information, but does not include exposure assessment information. The data in IRIS, combined with specific exposure information, can be used to help characterize the public health risks of a given situation. This risk characterization can then serve as input for a risk management decision designed to protect public health.

IRIS users are cautioned that quantitative risk estimates are subject to varying degrees of uncertainty and that the existence of such uncertainty should be taken into account in preparing site specific risk analyses. While the data base is updated monthly to reflect intra-Agency work group decisions, in some cases new health hazard data may have been generated on a particular substance since an on-line file was reviewed by the work groups. These data may not *yet* be reflected on the system, and should be considered in developing risk characterizations.

For more information on IRIS and access, contact:

Risk Information Hotline (Staffed by Labat-Anderson Inc.)
Environmental Criteria and Assessment Office
Office of Research and Development
U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, Ohio 45268 USA
Telephone: (513) 569-7254 FAX: (513) 569-7159

Office of Health and Environmental Assessment

Health risk assessment information on a chemical is included in IRIS only after a comprehensive review of chronic toxicity data by work groups composed of U.S. EPA scientists from several Program Offices. The summaries presented in Sections I and II represent a consensus reached in the review process. The other sections contain U.S. EPA information which is specific to a particular EPA program and has been subject to review procedures prescribed by that Program Office. The regulatory actions in Section IV may not be based on the most current risk assessment, or may be based on a current, but unreviewed, risk assessment, and may take into account factors other than health effects (e.g., treatment technology). When considering the use of regulatory action data for a particular situation, note the date of the regulatory action, the date of the most recent risk assessment relating to that action, and whether technological factors were considered. Background information and explanations of the methods used to derive the values given in IRIS are provided in the five Background Documents in Service Code 5, which correspond to Sections I through V of the chemical files.

STATUS OF DATA FOR Aroclor 1016

File On-Line 01/01/93

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	on-line	11/01/93
Inhalation RfC Assessment (I.B.)	no data	
Carcinogenicity Assessment (II.)	no data	
Drinking Water Health Advisories (III.A.)	no data	
U.S. EPA Regulatory Actions (IV.)	no data	
Supplementary Data (V.)	no data	

_I. CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

__I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2
Last Revised -- 11/01/93

The Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as carcinogenicity. In general, the RfD is an estimate

(with uncertainty spanning perhaps an order of magnitude) of a daily exposure
to the human population (including sensitive subgroups) that is likely to be
without an appreciable risk of deleterious effects during a lifetime. Please
refer to Background Document 1 in Service Code 5 for an elaboration of these
concepts. RfDs can also be derived for the noncarcinogenic health effects of
compounds which are also carcinogens. Therefore, it is essential to refer to
other sources of information concerning the carcinogenicity of this substance.
If the U.S. EPA has evaluated this substance for potential human carcinogen-
icity, a summary of that evaluation will be contained in Section II of this
file when a review of that evaluation is completed.

NOTE: A peer review of the non-cancer oral reference dose for Aroclor 1016,
to determine the adequacy of the studies underlying the reference dose for use
in risk assessments or otherwise, has been tentatively scheduled for December
1993.

___I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Reduced birth weights	NOAEL: 0.25 ppm in feed (0.007 mg/kg-day)	100	1	7E-5 mg/kg-day
Monkey Reproductive Bioassay	LOAEL: 1 ppm in feed (0.028 mg/kg-day)			
Barsotti and van Miller, 1984; Levin et al., 1988; Schantz et al., 1989, 1991				

*Conversion Factors: Dams received a total average intake of 4.52 mg/kg (0.25
ppm) or 18.41 mg/kg (1 ppm) throughout the 21.8-month (654 days) dosing
period. These doses are equivalent to 0.007 mg/kg-day and 0.028 mg/kg-day for
the identified NOAEL and LOAEL respectively.

___I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Barsotti, D.A. and J.P. van Miller. 1984. Accumulation of a commercial
polychlorinated biphenyl mixture (Aroclor 1016) in adult rhesus monkeys and
their nursing infants. Toxicology. 30: 31-44.

Levin, E.D., S.L. Schantz and R.E Bowman. 1988. Delayed spatial alternation
deficits resulting from perinatal PCB exposure in monkeys. Arch. Toxicol.
62: 267-273.

Schantz, S.L., E.D. Levin, R.E. Bowman et al. 1989. Effects of perinatal PCB
exposure on discrimination-reversal learning in monkeys. Neurotoxicol.
Teratol. 11: 243-250.

Schantz, S.L., E.D. Levin and R.E. Bowman. 1991. Long-term neurobehavioral
effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys.
Environ. Toxicol. Chem. 10: 747-756.

    These are a series of reports that evaluated perinatal toxicity and long-
term neurobehavioral effects of Aroclor 1016 in the same groups of infant

monkeys. Aroclor 1016 is a commercial mixture of polychlorinated biphenyls (PCBs) devoid of chlorinated dibenzofurans (Barsotti and van Miller, 1984). Analysis of the commercial feed used for this study revealed contamination with congeners specific for Aroclor 1248, present in the parts per billion range. These congeners were present in the control as well as test diets. Aroclor 1016 was administered to groups of 8 adult female rhesus monkeys via diet in concentrations of 0, 0.25 or 1.0 ppm for approximately 22 months. Based on a reported total Aroclor intake of 4.52 and 18.41 mg/kg over the 22-month exposure period (Schantz et al., 1989, 1991), the low- and high-doses are estimated to be 0.007 and 0.028 mg/kg-day, respectively. Exposure began 7 months prior to breeding and continued until offspring were weaned at age 4 months. No exposure-related effects on maternal food intake, general appearance, hematology, serum chemistry (SGPT, lipid, and cholesterol analyses) or number of breedings were observed (Barsotti and van Miller, 1984). All monkeys had uncomplicated pregnancies, carried their infants to term and delivered viable offspring. Teratologic examinations were not performed. Mean birth weights of the infants in the control, 0.007 and 0.028 mg/kg-day dose groups were 521 g, 491 g and 442 g, respectively (Barsotti and van Miller, 1984). The decrease in birth weight in the high-dose group was significantly (p<0.01) lower than in controls. Further statistical analysis of the infant birth weight data by the Agency indicated that gestation length did not significantly affect birth weight and the distribution of male and female infants in the various dose groups could not account for the difference in birth weights among the dose groups. Agency reanalysis of the data confirmed the significant decrease in body weight for the high-dose infants, although slightly different average values were obtained. Males that had sired some infants were exposed to Aroclor 1248, so the birth weight data were also analyzed excluding these infants. The results for this adjusted data indicated that control infants weighed 528 g, low-dose infants weighed 486 g, and high-dose infants weighed 421 g. Even with this adjustment there was still a significant difference (p<0.01) in birth weight for the high-dose group when compared with controls. No significant differences between treatment and control groups were detected in neonatal head circumference or crown-to-rump measurements. Both exposure groups showed consistent weight gains, but infant weights in the high-dose group were still lower (864 g) at weaning, although not significantly different from the controls (896 g). Hyperpigmentation was present at birth in the low- and high-dose infants but did not persist once dosing was stopped. This clinical change was determined not to be a critical adverse effect. The concentration of Aroclor 1016 in breast milk was higher than the maternal dose. No exposure-related hematologic effects were observed in the infants during the nursing period (Barsotti and van Miller, 1984). One of the offspring in the high-dose group went into shock and died on the day following weaning for unknown reasons (Schantz et al., 1989, 1991).

Behavioral testing of the infant monkeys was first performed at age 14 months and no overt signs of PCB toxicity were observed (Schantz et al., 1989, 1991). Two-choice discrimination-reversal learning was assessed using simple left-right spatial position, color and shape discrimination problems, with and without irrelevant color and shape cues. One of the offspring in the low-dose group stopped responding early in testing for an unknown reason and could not be induced to resume; therefore, test results were obtained using 6, 7 and 6 infants in the control, low- and high-dose groups, respectively. The offspring in the high-dose (0.028 mg/kg-day) group were significantly (p<0.05) impaired in their ability to learn the spatial position discrimination problem (i.e., achieved 9 correct choices in 10 trials), requiring more than 2.5 times as many trials as their age-matched controls. There were no significant learning differences between these groups on this problem during overtraining

(ability to achieve greater than or equal to 90% correct choices in two consecutive daily sessions) or position reversals. The only other exposure-related effect was significantly facilitated learning ability (p<0.05) on the shape discrimination problem at 0.028 mg/kg-day.

Performance on delayed spatial alternation (a spatial learning and memory task) was assessed in the offspring monkeys at age 4-6 years (Levin et al., 1988; Schantz et al., 1991). The two Aroclor-exposed groups were not significantly different from controls (p<0.05) in test performance. However, the exposed groups did significantly (p<0.05) differ from each other. The difference between the two exposed groups was due to a combination of facilitated performance at the low-dose (0.007 mg/kg-day) and impaired performance at the high-dose (0.028 mg/kg-day). Although these data are insufficient for establishing an exposure-effect relation due to the lack of difference between exposed and control groups, the investigators suggested that the performance deficit at 0.028 mg/kg-day may have been exposure-related. The investigators noticed that a paradoxical biphasic effect occurred on the same test when comparing low-dose and high-dose infants. This same effect has been observed for lead-exposed monkeys.

To summarize the above, adult monkeys that ingested 0.007 or 0.028 mg/kg-day doses of Aroclor 1016 for approximately 22 months showed no evidence of overt toxicity. Effects occurring in the offspring of these monkeys consisted of hairline hyperpigmentation at greater than or equal to 0.007 mg/kg-day, and decreased birth weight and possible neurologic impairment at 0.028 mg/kg-day. Based on the reduced birth weights of prenatally-exposed monkeys, the 0.007 mg/kg-day dose is the NOAEL and the 0.028 mg/kg-day dose is a LOAEL in monkeys.

The results of the neurobehavioral tests in the monkey offspring at 14 months and 4-6 years of age indicate adverse learning deficits at the 0.028 mg/kg-day maternal dose. Evaluation of these data is complicated by possible inconsistencies in the outcome of both the discrimination-reversal learning tests (learning was impaired and facilitated on different problems) and the delayed spatial alternation test (performance significantly differed between the two exposed groups, but not between either test group and the control). However, there is evidence suggesting that deficits in discrimination-reversal learning and delayed spatial alternation are related to decreased brain dopamine (Schantz et al., 1991), which has been observed in monkeys orally exposed to Aroclor 1016 (Seegal et al., 1990, 1991). Behavioral dysfunctions, including deficits in visual recognition and short-term memory, also have been observed in infants of human mothers who consumed fish contaminated with PCB mixtures of unknown composition (Fein et al., 1984a,b; Jacobsen et al., 1985, 1990; Gladen et al., 1988).

___I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF -- A 3-fold factor is applied to account for sensitive individuals. The results of these studies, as well as data for human exposure to PCBs, indicate that infants exposed transplacentally represent a sensitive subpopulation. A factor of 3 is applied for extrapolation from rhesus monkeys to human. A full 10-fold factor for interspecies extrapolation is not considered necessary because of similarities in toxic responses and metabolism of PCBs between monkeys and humans and the general physiologic similarity between these species. In addition, the rhesus monkey data are predictive of other changes noted in human studies such as chloracne, hepatic changes, and effects on

reproductive function. A factor of 3 is applied because of limitations in the data base. Despite the extensive amount of animal laboratory data and human epidemiologic information regarding PCBs, the issue of male reproductive effects is not directly addressed and two-generation reproductive studies are not available. As the study duration was considered as somewhat greater than subchronic, but less than chronic, a partial factor of 3 is used to account for extrapolation from a subchronic exposure to a chronic RfD.

MF -- None

___I.A.4. ADDITIONAL STUDIES / COMMENTS (ORAL RfD)

Male pig-tailed macaques [Macaca nemistrina], (number not reported, age 3-7 years, 5-9 kg initial body weight) were administered Aroclor 1016 dissolved in corn oil on bread in doses of 0, 0.8, 1.6 or 3.2 mg/kg-day for 20 weeks (Seegal et al., 1991). There were no overt signs of intoxication or exposure-related effects on body weight gain. Neurochemical analyses of various regions of the brain were performed following termination of exposure. Dose-related decreased concentrations of dopamine were observed in the caudate nucleus, putamen, substantia nigra, and hypothalamus, but not in the globus pallidus or hippocampus. There were no exposure-related changes in concentrations of norepinephrine, epinephrine, or serotonin. Other neurologic endpoints were not evaluated.

Subchronic oral studies of Aroclor 1016 have been performed in species other than monkeys. These species were tested at doses higher than the 0.007 and 0.028 mg/kg-day doses fed to monkeys in the principal studies.

Groups of 10 female Sprague-Dawley rats (age not reported, body weight approximately 225-250 g at start) were fed 0, 1, 5 or 50 ppm Aroclor 1016 in the diet for 5 months (Byrne et al., 1988). The Aroclor was dissolved in acetone that was evaporated from the diet prior to feeding. Using a rat food consumption factor of 0.05 kg food/kg bw (U.S. EPA, 1987), the doses are estimated to be 0, 0.05, 0.25 and 2.5 mg/kg-day. Serum levels of adrenal cortical hormones were evaluated four times throughout the treatment period. Adrenal dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHS) levels were significantly ($p < 0.05$) reduced at all treatment levels after approximately 100 days of exposure. Serum corticosterone (the principal glucocorticoid in rats), adrenal weight, adrenal histology, and nonadrenal endpoints other than food consumption were not evaluated. Food consumption did not significantly differ between and among control and treatment groups. Because insufficient information is available to determine whether the decreases in circulating adrenal hormones were physiologically significant, it is uncertain whether the doses are NOAELs or LOAELs for Aroclor 1016 in rats.

Male Balb/c mice (18-20 g body weight) were fed Aroclor 1016 mixed in diet at concentrations of 0 or 5 ppm for 3 or 6 weeks (Loose et al., 1978). Using a mouse food consumption factor of 0.13 kg food/kg bw (U.S. EPA, 1987), the dose is estimated to be 0.65 mg/kg-day. Sensitivity to Salmonella typhosa endotoxin (15 mice per endotoxin dose) and resistance to infection by Plasmodium berghei (malaria parasitemia; number of mice not reported) were evaluated. Sensitivity to the endotoxin was significantly ($p < 0.05$) increased after 3 weeks of exposure as indicated by endotoxin LD50 values of 152 and 844 ug in the Aroclor-exposed and control groups, respectively. Sensitivity to the endotoxin after 6 weeks of Aroclor exposure was not evaluated. There were no significant ($p < 0.05$) effects of Aroclor exposure for 3 or 6 weeks on

malaria lethality as indicated by post-inoculation surv_val time. No other endpoints were evaluated in this study. When injected into neonates, splenic cells from C57Bl/6 male mice exposed to 167 ppm (21.71 mg/kg-day) dietary Aroclor 1016 for 3 weeks elicited a greater graft-versus-host reaction than controls (Silkworth and Loose, 1978). Based on the decreased resistance to infection leading to death, 0.65 mg Aroclor 1016/kg-day suggests a LOAEL for immunotoxicity for subchronic exposure in male mice.

Aulerich and Ringer (1977) performed a breeding study in which groups of 8 female and 2 male adult pastel mink were fed diets containing 0 or 2 ppm Aroclor 1016 for 39 weeks or until the kits were 4 weeks of age. The Aroclor was dissolved in acetone which was evaporated from the diet prior to feeding. Using assumed values of 150 g/day for food consumption and 0.8 kg for body weight for female mink (Bleavins et al., 1980), the estimated dose of Aroclor 1016 is 0.4 mg/kg-day. Monthly determinations showed no statistically significant differences (p<0.05) between the control and treated mink in body weight gain, hemoglobin, and hematocrit. Additionally, tabulated data showed no treatment-related effects on female survival, numbers of females mated, number of females that gave birth, number of kits born alive or dead, number of births per female, average birth weight or number of kits alive at 4 weeks. The evidence for lack of treatment-related effects on body weight, hematology, reproduction and survival suggests that 0.4 mg/kg-day is a NOAEL for Aroclor 1016 in mink.

Groups of adult Pastel mink were fed a diet containing 0 ppm (24 females and 6 males) or 20 ppm (12 females and 3 males) Aroclor 1016 during a 247-day breeding study (Bleavins et al., 1980). Aroclor was dissolved in acetone which was evaporated from the diet prior to feeding. Using assumed values of 150 g/day for food consumption and 0.8 kg for body weight for female mink reported by the investigators, the estimated dose of Aroclor 1016 is 3.8 mg/kg-day. There were no deaths in the exposed or control males. Mortality was higher in the exposed females [25% (3/12) compared with 12.5% (3/24) in controls], but no clear difference in survival time was observed. Necropsies for gross abnormalities were performed on all control and treated mink that died; these showed effects only in the treated mink consisting of emaciation characterized by an almost complete absence of body fat. Histologic examinations were not performed. The incidence of mated females giving birth was reduced in the exposed group [44.4% (4/9) compared with 76.2% (16/21) in controls], but average gestation length, live births and birth weight did not significantly differ (p>0.05) between exposed and control groups. Body weight at age 4 weeks, average number of infants per lactating female and infant biomass (average body weight gain through age four weeks x average number of infants raised per lactating female) were significantly (p<0.05) reduced in the exposed group. Mortality during the first 4 weeks of life was increased in the exposed group [56.0% (14/25) compared with 24.1% (19/79) in controls]. The investigators noted that the adverse effects on reproduction do not appear to be due to an effect on spermatogenesis, since PCB-treated male mink have had acceptable levels of reproduction when mated to untreated females in other studies. The evidence for impaired reproduction and increased maternal and postnatal mortality suggests that 3.8 mg Aroclor 1016/kg-day is an FEL in mink. Although the FEL from this study and NOEL of 0.4 mg/kg-day from Aulerich and Ringer (1977) suggest that the dose-severity slope for Aroclor 1016 in mink is steep, neither study tested sufficient numbers of animals or dose levels to allow definitive conclusions to be drawn.

Dermal lesions including skin irritation, chloracne and increased pigmentation of skin and nails have been observed in humans occupationally exposed to Aroclor 1016 and other Aroclor formulations by both inhalation and

dermal routes (Fischbein et al., 1979, 1982, 1985; Ouw _c al., 1976; Smith et al., 1982). However, insufficient data are available to determine possible contributions of Aroclor 1016 alone, extent of direct skin exposure and possible contaminants in these occupational studies.

Decreased birth weight has also been reported in infants born to women who were occupationally exposed to Aroclor 1016 and other Aroclor formulations (Taylor et al., 1984, 1989), ingested PCB-contaminated fish (Fein et al., 1984a,b) and ingested heated Kanechlor PCBs during the Yusho and Yu-Cheng incidents (Rogan, 1989; Yamashita, 1977). Due to uncertainties regarding actual sources of PCB exposure, and other confounding factors and study limitations, the decreases in human birth weight cannot be solely attributed to PCBs, particularly specific PCB mixtures. However, due to the consistency with which the effect has been observed, the human data are consistent with the Aroclor 1016-induced decreased birth weight in monkeys reported in the principal studies.

The human data available for risk assessment of Aroclor 1016 are useful only in a qualitative manner. Studies of the general population exposed to PCBs by consumption of contaminated food, particularly neurobehavioral evaluations of infants exposed in utero and/or through lactation, have been reported, but the original PCB mixtures, exposure levels and other details of exposure are not known (Kreiss et al., 1981; Humphrey, 1983; Fein et al., 1984a,b; Jacobson et al., 1984a, 1985, 1990a,b; Rogan et al., 1986; Gladen et al., 1988). Most of the information on health effects of PCB mixtures in humans is available from studies of occupational exposure. Some of these studies examined workers who had some occupational exposure to Aroclor 1016, but in these studies concurrent exposure to other Aroclor mixtures nearly always occurred, exposure involved dermal as well as inhalation routes (the relative contribution by each route was not known), and monitoring data were lacking or inadequate (Fischbein et al., 1979, 1982, 1985; Fischbein, 1985; Warshaw et al., 1979; Smith et al., 1982; Lawton et al., 1985).

Information specifically on the oral absorption of Aroclor 1016 is not available, but studies of individual congeners and PCB mixtures of higher chlorine content in animals indicate, in general, that PCBs are readily and extensively absorbed. These studies have found oral absorption efficiency on the order of 75 to >90% in rats, mice, monkeys and ferrets (Albro and Fishbein, 1972; Allen et al., 1974; Tanabe et al., 1981; Bleavins et al., 1984; Clevenger et al., 1989). A study of a PCB mixture containing 54% chlorine provides direct evidence of absorption of PCBs in humans after oral exposure (Buhler et al., 1988), and indirect evidence of oral absorption of PCBs by humans is available from studies of ingestion of contaminated fish by the general population (Schwartz et al., 1983; Kuwabara et al., 1979). There are no quantitative data regarding inhalation absorption of PCBs in humans but studies of exposed workers suggest that PCBs are well absorbed by the inhalation and dermal routes (Maroni et al., 1981a,b; Smith et al., 1982; Wolff, 1985). PCBs distribute preferentially to adipose tissue and concentrate in human breast milk due to its high fat content (Jacobson et al., 1984b; Ando et al., 1985).

The metabolism of PCBs following oral and parenteral administration in animals has been extensively studied and reviewed, but studies in animals following inhalation or dermal exposure are lacking (Sundstrom and Hutzinger, 1976; Safe, 1980; Sipes and Schnellmann, 1987). Information on metabolism of PCBs in humans is limited to occupationally exposed individuals whose intake is derived mainly from inhalation and dermal exposure (Jensen and Sundstrom, 1974; Wolff et al., 1982; Schnellmann et al., 1983; Safe et al., 1985; Fait et

al., 1989). In general, metabolism of PCBs depends on the number and position of the chlorine atoms on the phenyl rings of the constituent congeners (i.e., congener profile of the PCB mixture) and animal species. Although only limited data are available on metabolism of PCBs following inhalation exposure, there is no reason to suspect that PCBs are metabolized differently by this route.

Data exist on the in vitro hepatic metabolism and in vivo metabolic clearance of 2,2',3,3',6,6'-hexachlorobiphenyl and 4,4'-dichlorobiphenyl congeners in humans, monkeys, dogs, and rats (Schnellmann et al., 1985). Both of these congeners are present in Aroclor 1016, but the hexachlorobiphenyl is only a minor constituent. For each congener, the Vmax values for metabolism in the monkey, dog and rat are consistent with the respective metabolic clearance values found in vivo. Thus, the kinetic constants for PCB metabolism obtained from the dog, monkey, and rat hepatic microsomal preparations were good predictors of in vivo metabolism and clearance for these congeners. In investigations directed at determining which species most accurately predicts the metabolism and disposition of PCBs in humans, the in vitro metabolism of these congeners was also studied using human liver microsomes (Schnellmann et al., 1983, 1984). Available data suggest that metabolism of PCBs in humans most closely resembles that of the monkey and rat. For example, the in vitro apparent Km and Vmax for humans and monkeys are comparable. These studies show consistency between the in vitro and in vivo findings and collectively indicate that metabolism of the two congeners is similar in monkeys and humans.

___I.A.5.   CONFIDENCE IN THE ORAL RfD

Study -- Medium
Data Base -- Medium
RfD -- Medium

Confidence in the critical studies is rated medium since essentially only one group of monkeys has been examined. The initial study was well conducted in a sensitive animal species (rhesus monkeys) that closely resembles humans for many biological functions. These studies evaluated many sensitive endpoints of PCB toxicity and the effects observed have also been documented for human exposure. Many sophisticated reproductive and neurologic tests were performed over 6 years and many clinical chemistry determinations were conducted on the dams during the exposure period. Very extensive analyses of feed samples and tissue samples from dosed monkeys were performed. Although contamination of the control laboratory primate diet with PCBs other than those found in Aroclor 1016 was detected, the level of contamination was at the level of parts per billion and dosing of Aroclor 1016 was in the parts per million range. Because the contamination was consistent across all treatment groups and controls, quantitative comparison of adverse effects can be made. The investigators carefully documented the levels of test material and contaminant throughout the exposure and post-exposure period in animal tissues. Because the system of placentation, hemothelial-chorial with bidiscoidal distribution, is similar for Rhesus monkeys and humans, it is felt that toxic events that are induced during gestation for Rhesus monkeys will be highly predictive of similar events in humans. Historically, developmental neurobehavioral effects observed in rhesus monkeys are predictive of similar effects in humans. Although these studies were performed in an academic setting prior to the era of Good Laboratory Practices- Quality Control-Quality Assurance, the study report provides ample documentation of the experimental

protocol and quality of data collected. While the group sizes for this study are small (8 monkeys/group) when compared with the standards for rodent studies they are within the acceptable range for studies of large mammalian species as determined by EPA.

The data base for PCBs in general is extensive. Studies examining Aroclor 1016 have been performed in rhesus monkeys, mice, rats and mink. However, despite the extensive amount of data available only medium confidence can be placed in the data base at this time. It is acknowledged that mixtures of PCBs found in the environment do not match the pattern of congeners found in Aroclor 1016, therefore the RfD is only given medium confidence. For those particular environmental applications where it is known that Aroclor 1016 is the only form of PCB contamination, use of this reference dose may rate high confidence. For all other applications only medium confidence can be given. The U.S. EPA recognizes that there is a diversity of opinion among scientists concerning the use of the monkey studies for determining PCB toxicity. However, all of the studies in the vast data base for this chemical mixture support the conclusions reached in this document.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document -- This assessment is not presented in any existing U.S. EPA document.

Other EPA Documentation -- U.S. EPA, 1980, 1984, 1989, 1990

Agency Work Group Review -- 02/21/90, 03/25/92, 06/23/92, 09/24/92, 10/15/92, 11/04/92, 02/11/93

Verification Date -- 11/04/92

___I.A.7. EPA CONTACTS (ORAL RfD)

John L. Cicmanec / OHEA -- (513)569-7481

Michael L. Dourson / OHEA   (513)569-7531

__I.B. REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2

Not available at this time.

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2

This substance/agent has not been evaluated by the U.S. EPA for evidence of
human carcinogenic potential.

_III. HEALTH HAZARD ASSESSMENTS FOR VARIED EXPOSURE DURATIONS

__III.A. DRINKING WATER HEALTH ADVISORIES

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2

Not available at this time.

__III.B. OTHER ASSESSMENTS

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2

Content to be determined.

_IV. U.S. EPA REGULATORY ACTIONS

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2

Not available at this time.

_V. SUPPLEMENTARY DATA

Substance Name -- Aroclor 1016

Not available at this time.

_VI. BIBLIOGRAPHY

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2   ᗡ
Last Revised -- 02/01/93

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__VI.B.   INHALATION RfD REFERENCES

None

__VI.C.   CARCINOGENICITY ASSESSMENT REFERENCES

None

__VI.D.   DRINKING WATER HA REFERENCES

None

_____

_VII.   REVISION HISTORY

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2

Date	Section	Description
08/01/91	I.A.	Oral RfD now under review
08/01/92	I.A.	Work group review dates added
10/01/92	I.A.	Work group review date added
12/01/92	I.A.	Work group review dates added
01/01/93	I.A.	Oral RfD assessment on-line
01/01/93	VI.A.	Oral RfD references on-line
02/01/93	VI.A.	Oral RfD references corrected
03/01/93	I.A.6.	Work group review date added
09/01/93	I.A.	Oral RfD noted as going to be externally peer reviewed
11/01/93	I.A.	Note revised

SYNONYMS

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2
Last Revised -- 01/01/93

12674-11-2
AROCLOR 1016
HSDB 6352

JAN 12 1993

Mr. Stephen B. Hamilton, Jr.
Manager, Environmental Science and Technology
General Electric Company
3135 Easton Turnpike
Fairfield, CT 06431

Dear Mr. Hamilton:

I am writing to follow up with you regarding issues raised at our meeting of January 6, 1993, where we discussed the Environmental Protection Agency's (EPA) Reference Dose (RfD) for Aroclor 1016. As I indicated to you at the meeting, we share your concern in assuring that the RfD for Aroclor 1016 is based on sound science. Considering the issues raised at the meeting, I believe that a peer review of these data would be appropriate.

I have asked ORD's Risk Assessment Forum to conduct this peer review in the near future. The peer review will focus on the Wisconsin primate data on which the calculation of the RfD for Aroclor 1016 was primarily based, and will also examine other data relevant to this RfD as it appears on the Integrated Risk Information System (IRIS), as well as the background documents. Any additional information that you might be able to provide to the Agency relevant to the RfD for Aroclor 1016 will be appreciated. This information and any questions you might have should be directed to Dr. Dorothy Patton, Director of the Risk Assessment Forum, at (202) 260-6743.

Sincerely yours,

Erich W. Bretthauer
Assistant Administrator
for Research and Development

cc: F. Henry Habicht II
       William Farland
       Dorothy Patton

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
CINCINNATI. OHIO 45268

January 28, 1993

MEMORANDUM

SUBJECT: Change in NOAEL for Aroclor 1016 from verified
           Concentration

FROM: John L. Cicmanec, D.V.M.
           RfD/RfC Work Group Member

TO: RfD/RfC Work Group

     During the process of final editorial review of the Aroclor
1016 file, just before it was loaded onto IRIS, Gary Foureman
discovered that we had erroneously transposed the dosage of 0.008
mg/kg-day as the NOAEL dose instead of the **correct** value of 0.007
mg/kg-day. The 0.008 mg/kg-day is used in the earliest published
paper of the series of key references Barsotti, D.A. and Van
Miller, J. P. "Accumulation of a commercial polychlorinated
biphenyl mixture (Aroclor 1016) in adult rhesus monkeys and their
nursing infants", Toxicology 30:31-44, 1984. The **correction to** the
value of 0.007 mg/kg-day was made in the Schantz paper, Schantz,
S. L., Levin, E. D., Bowman, R. E., Heironimus, M. P., and
Laughlin, N. K., "Effects of perinatal PCB exposure on
discrimination-reversal learning in monkeys", Neurotox. and
Teratol. 11:243-250, 1988. The change resulted from the
investigators taking a second, more critical, look at the actual
dosing information.

     This matter is being brought to the attention of the entire
Work Group for the purpose of providing formal documentation of the
change as it will appear in the meeting notes.

Stephen D. Ramsey
Vice President-Corporate Environmental Programs

July 30, 1992

Mr. F. Henry Habicht, II
Deputy Administrator
U. S. Environmental Protection Agency
401 M Street SW
Washington, DC 20460

Dear Hank:

We understand that EPA is considering establishing a non-cancer reference
dose (Rfd) for PCB Aroclor 1016 in the IRIS database in August or September.
Currently, no Rfd exists for non-cancer effects and EPA's standard setting and
risk assessment is driven solely by the cancer risk attributed to PCBs by EPA.
GE's scientists tell me that this new Rfd would in most instances result in
clean up standards for PCBs which are even more stringent than those
currently established using cancer toxicity as the primary risk driver.

We are concerned that adding a non-cancer Rfd to IRIS is ill-considered and
premature and ask that you have your staff reconsider this proposed action. In
this regard, we request a meeting with you and appropriate members of your
staff to outline our specific concerns before EPA takes any action.
Summarized below are some of the numerous factors which militate in favor of
much more careful consideration by EPA:

● The Administrator has told us that a comprehensive reassessment of
how PCBs are regulated is underway. Indeed, that reassessment appears
to be progressing at a deliberate but slow pace considering the
convincing nature of new evidence on PCB cancer toxicity. The
introduction of a non-cancer Rfd during the interim appears to defeat
the purpose and predetermine the outcome of the comprehensive
reassessment. Moreover, the speed with which this is occurring
contrasts with the much slower pace of the general PCB reassessment.

● You have directed a thorough review of how the IRIS process can be
improved. None of the process changes now being considered by the
Agency, e.g. public participation, peer review and better expressions of
uncertainties, were used in establishing the proposed Rfd for PCBs. It is
antithetical to your attempt to open the IRIS process to hurriedly create
an Rfd for PCBs, which is one of the most scientifically controversial
and environmentally ubiquitous chemicals, before the process
improvements you seek are implemented.

● You have also directed a shift away from single-number risk assessments
to a broader approach of risk management that considers the

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uncertainties of the scientific information available and a variety of
exposure scenarios. A single number Rfd reverts the regulatory
approach for PCBs back to policy by the numbers and will result in the
risk assessment process not taking into account more real world, actual
risks.

● The animal studies that will be relied upon for the Aroclor 1016 Rfd and
other PCB Rfds have been criticized in the scientific community. As
stated above, the process used in this instance allowed little opportunity
for a thorough and open review. In addition, the Agency has no
guidelines for measuring many of the health effects that would be
considered for other Aroclor Rfds, e.g. immunotoxicity. These effects
should not be measured and translated into IRIS standards until there is
scientific agreement on the measurements and the real concerns raised
by the criticisms of the studies have been resolved. In short, what is the
rush here?

● Finally the proposed Rfd would be inconsistent with risk standards set
by other agencies, e.g. FDA. We are unaware of any significant
interagency coordination on this issue.

Hank, I believe that top EPA management attention to this important Agency
action is warranted to insure that good science is translated into good policy.
I have asked Dr. Steve Hamilton of my staff to meet as soon as possible with
Erich Bretthauer on the science issues. I will call you shortly to set a meeting
date for us to discuss the science as well as the policy implications.

Sincerely,

Stephen D. Ramsey

SDR/an

cc: E. Bretthauer
      R. Guimond

Stephen D. Ramsey
Vice President-Corporate Environmental Programs

General Electric Company
3135 Easton Turnpike, Fairfield, CT 06431
203 373.3067

September 3, 1992

Dr. F. Henry Habicht II
Deputy Administrator
U.S. Environmental Protection Agency
401 M Street SW
Washington, DC 20460

Dear Hank:

I wanted to follow-up my letter to you of July 30, 1992 concerning EPA's plans to
establish a new non-cancer reference dose (RfD) for PCB Aroclor 1016. Members
of Erich Bretthauer's staff met with Dr. Steve Hamilton and Ms. Marion
Herrington of my staff on August 20. While the meeting was informative and
candid, and much appreciated by us, we continue to be very concerned with the
Agency's plans to establish the RfD in the IRIS database at a time when
reevaluation of the IRIS process is ongoing but has not yet been completed. This
is particularly the case when the data and studies upon which this action will be
based are seriously flawed. Because this issue is so important to GE, to EPA and
to the public, I ask that before a final decision is reached, we have an opportunity
to meet with you to give you our reasons why a decision at this time would be
premature and ill-advised. I've summarized our concerns below.

As we understand it, EPA is planning to establish a reference dose (RfD) of 8 X 10
(-5) mg/kg/day for Aroclor 1016 based on reproductive studies in rhesus
monkeys carried out by Drs. James Allen, D. Barsotti and colleagues at the
University of Wisconsin - Madison in the 1970s. At the meeting of August 20,
Dr. Hamilton outlined seven examples of known dosing and/or cross
contamination problems that demonstrate the unreliable nature of the chemical
handling and dose administration practices that were employed in those studies.
Chemicals involved in these studies included various Aroclor PCB mixtures,
polybrominated biphenyls and TCDD. For example, one third of the test group
receiving Aroclor 1016 had to be dropped from the published account of this
work because the animals received polybrominated biphenyls as well as PCB.
The remaining test population, as well as some of the controls, also received
some level of Aroclor 1248, the Aroclor mixture containing the highest
concentration of TCDD equivalents. As a result of this cross-contamination,
strong peaks were observed in the gas chromatograms from tissue samples of the
Aroclor 1016 study representing "dioxin-like" congeners that are not present in
1016. We believe that the presence of these congeners can account for the

anomalous dioxin-like toxic effects and the reproductive effects observed in the offspring of the test animals. Alternatively, one cannot rule out the presence of dioxin, itself, possibly resulting from cross-contamination from a concurrently run experiment.

In addition, PCBs quantitated as Aroclor 1016 were found in tissue samples taken from five of 8 randomly selected animals before the experiment began. Therefore, we maintain that it is impossible to determine from these tests the identity of the toxicant(s) or the dose at which an effect was observed. A reference dose based upon such data would be meaningless.

Members of your staff indicated that they were familiar with some of the problems that we described, but had discounted their significance. Part of their reasoning was that the reference dose calculated for 1016 was similar to that calculated for Aroclor 1248 based on work by the same investigators. Since the 1248 study suffered from similar quality problems as the 1016 study, a similar result is not surprising and certainly does not justify the use of bad data. We obviously strenuously disagree about the significance of these flaws in the ways the studies were conducted. Many toxicologists who are familiar with the field of halogenated hydrocarbon toxicological research know about the problems with the rhesus monkey studies carried out at the University of Wisconsin in the 1970s. Several notable scientists have expressed their disbelief on learning that EPA plans to use this work as a basis for quantitative risk assessment.

Aside from the suspect quality of the data, we are also concerned about the approach the Agency has proposed to take in applying uncertainty factors to derive the RfD for Aroclor 1016. At the August 20 meeting, we learned for the first time of the assumptions to be used to account for uncertainties associated with the primate studies. Serious questions arise about the appropriateness of using certain of these safety factors that we would like to discuss further with your staff before any decision is made to finalize the proposed Aroclor 1016 RfD.

I find it very disturbing that the plan to establish the 1016 RfD is occurring at a time when the IRIS process is undergoing review to determine how it can be improved through public participation, peer review and other mechanisms that will ensure an open and thorough review of the effects of chemical substances. Given the amount of criticism the scientific community has leveled against the University of Wisconsin studies, it is especially troubling that the Agency is moving forward with establishment of the 1016 RfD before review of the IRIS process has been completed.

As you are aware, once EPA establishes a reference dose for PCBs, it will be virtually impossible to alter it. States will follow EPA's lead in their risk assessment approaches. It will become a regulatory totem which will be virtually untouchable. This decision is going to drive very costly clean-up and compliance decisions which will result in the expenditures of millions and perhaps billions

of dollars by American companies. Great care must be taken to make sure such a decision is the right one, based on unquestionable science, not rushed to decision on a flawed record.

Given the importance of the establishment of this RfD to the regulated community, the apparent impasse in discussions between GE and EPA scientists on substantial scientific issues and EPA's plans for improving the IRIS review process, I request that EPA place the establishment of the RfD for Aroclor 1016 on hold until a thorough review of PCB risk science can be completed and presented to EPA's Science Advisory Board.

Finally, on a related matter, my staff was told that the requested re-evaluation of cancer potency factors for PCBs, based on the data from the re-read of the liver tumor slides performed by the Institute for Evaluating Health Risks (IEHR), is not going forward. Dr. Dorothy Cantor, an assistant to Don Clay, acknowledged in a meeting in February, 1992 involving GE and Region II that EPA considers the re-read to be of high quality, the result of a process overseen by EPA and other government scientists. Administrator Reilly has on several occasions told G.E.'s Chairman that such a review was in fact underway. We would appreciate knowing the status of the Agency's evaluation of PCBs. If no such review is to be performed, it is even more alarming to us that EPA would move rapidly to develop a non-cancer reference dose based on low quality data while not moving forward with an evaluation of cancer potency factors which is based on high quality data.

I understand that the EPA staff will consider further the scientific points reviewed by us and that the earliest entry of the Aroclor RfD into the IRIS data base is now October 1, 1992. I plan to be out of the country until the first week in October. If you feel you must proceed with the October 1 entry, I request that you meet with members of my staff in my absence. The optimum time for me to meet with you is October 15 or 16 in Washington.

Mr. Larry Boggs of my staff will be contacting you regarding a meeting time.

Sincerely yours,

Stephen D. Ramsey

SDR/bjb

Bacon & Bernz
ATTORNEYS AT LAW

10 Little Britain Road
Newburgh, N.Y. 12550

• (914) 569-8010   FAX: (914) 562-5271 •

JAMES B. BACON   DAVID S. BERNZ

October 15, 1992

William S. Reilly, Administrator
USA EPA
410 Main Street. N.W.
Room 1200, West Tower
Washington, D.C. 20460

Re: Superfund review of PCB contamination in the Hudson
River.

Dear Mr. Reilly,

I write to urge you to have EPA's Environmental
Criteria and Assessment Office promulgate a Reference Dose
Value for the non-carcinogenic health effects of PCBs as
soon as possible. This number must be assigned in time to
be included in the current reassessment of the PCB problem
in the Hudson River by EPA Region 2.

As you know, the General Electric Company has been
identified as the responsible party for the federal Hudson
River PCB Superfund site which is on the national priorities
list. Throughout, G.E. has attempted to manipulate the
reassessment process and now seeks to avoid inclusion of the
non-cancer risks of PCBs in the ongoing reassessment. I am
particularly disturbed by G.E's exertion of political
influence over the EPA reassessment far outside the
legitimate processes provided for expressing its concerns.

No doubt, G.E. hopes to avoid liability by delaying or
weakening the reassessment process until a flood or other
event spreads the PCB hot-spots and makes a clean-up
impracticable. G.E.'s back-door influence is unconscionable
and must not be allowed to prevent EPA from doing its job.
The public interest requires inclusion of the non-cancer
risks in EPA's assessment as many people continue to eat
fish caught in the Hudson River.

Furthermore, If G.E. is permitted to exert undue
influence to escape liability here, it will only make EPA's
job more difficult when attempting to hold G.E. and
responsible parties accountable elsewhere.

As an attorney with some limited experience in
environmental law, I am keenly aware of the complexity of
the issues involved when dealing with the liability of a

large company for a toxic clean-up. Yet the public deserves, and the law provides for a PCB clean-up of the Hudson River paid for by the responsible party. Anything you can do to expedite this will be greatly appreciated by many.

Thanks, in advance for your time and consideration.

Yours truly,

David Bernz, Esq.

cc: Hon. Hamilton Fish, Member of Congress
Hudson River Sloop Clearwater, Inc.

October 28, 1992

William Reilly, Administrator
USA EPA
410 Main St. N.W.
Rm. 1200, West Tower
Washington, D.C. 20460

Dear Mr. Reilly:

We, the undersigned, include organizations and individuals who have maintained a long-standing commitment to the remediation of the PCB problem that plagues the Hudson River. We are writing to you at this time to express our extreme concern regarding the efforts of the General Electric Co. (G.E.) to influence EPA's Superfund review of this site.

As you know, G.E. has been identified as the responsible party for the federal Hudson River PCB Superfund site which is on the National Priorities list. It has now come to our attention that G.E. is attempting to exert pressure on the federal EPA in an effort to derail the promulgation of a numerical value (Reference Dose Value or RfD) for the non-carcinogenic health effects of PCBs by EPA's Environmental Criteria and Assessment Office (ECAO).

At this time, the EPA Region 2 office is in the midst of the second phase of a Remedial Investigation/Feasibility Study for this site, which includes a human health risk assessment. Region 2 has indicated that it intends to include non-carcinogenic

toxicities of PCBs in its assessment, providing that ECAO is able to provide a numerical value before the review is completed.

Clearly, it is in G.E.'s interest to delay ECAO approval of an RfD for PCBs, in an attempt to prevent this information from being factored into the decision for the Hudson River Superfund site. However, it is clearly not in the public's interest that sound scientific information which is critical to this decision be omitted. Further, because PCBs are the tenth most common contaminant at federal Superfund sites, and the priority pollutant at 185 sites, the negative implications of G.E.'s actions are far reaching.

It is of utmost importance that EPA not deviate from its normal procedures for promulgation and incorporation of a toxicity value into the Integrated Risk Information System (IRIS) database. If G.E. has legitimate questions or concerns regarding the numerical value EPA derives, federal regulations (Federal Register Vol. 53, No. 106, 6/2/88) provide a clear process through which the concerns of outside parties can be raised and evaluated, and an appropriate response made.

A report by the U.S. Office of Technology Assessment ("Coming Clean, Superfund Problems Can Be Solved", 1989) criticized EPA for allowing responsible parties to influence Superfund decisions. G.E, which is responsible for over 50 federal Superfund sites, more than any other corporate polluter,

has been particularly aggressive in seeking to influence public opinion, elected officials, public agencies, the press and the scientific community in relation to the Hudson River PCB Superfund site.

For over 20 years, the Hudson River and people along its shores have suffered the impacts of extensive PCB contamination. We urge EPA to act without further delay, to adopt a value for the non-cancerous health effects of PCBs, so that EPA's ultimate decision on the Hudson River can be one based on sound science and responsible public policy.

Sincerely,

Bridget Barclay
Hudson River Sloop Clearwater, Inc.

Sarah Clark
Environmental Defense Fund

Derry Bennett
American Littoral Society

Lee Wasserman
Environmental Planning Lobby

Jane Nogaki
N.J. Environmental Federation

Ann Rabe
Citizens Environmental Coalition

Sarah Chasis
Natural Resources Defense Council

Cara Lee
Scenic Hudson, Inc.

Leona Hoodes
Orange Environment

Sarah Meyland
Citizens Campaign

David Miller
National Audubon Society

Tom Lake
Fisherman

Cindy Zipf
Clean Ocean Action

Barnabus McHenry

cc: Congresswoman Nita Lowey
     Congressman Hamilton Fish, Jr.
     Congressman George Hochbrueckner
     Senator Daniel P. Moynihan
     NYS Attorney General Robert Abrams
     NYS Assemblyman Maurice Hinchey
     NYS Assemblyman George Pataki
     NYS Senator Mary Goodhue
     Congressman Matthew McHugh
     Thomas Jorling, Commissioner of Environmental Conservation
     Dr. Andrew Carlson, NYS Dept. of Health
     Constantine Sidamon-Eristoff, EPA Region 2 Adminstrator
     Eric Bretthauer, Assistant Administrator, EPA Office of
                    Research and Development
     F. Henry Habicht, EPA Deputy Administrator

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

May 25, 1990

SUBJECT: RfD Meeting - February 21, 1990

FROM: Mike Dourson
       Office of Technology Transfer and Regulatory Support

TO: RfD Work Group

Chemical Name: Aroclor 1016   (Oral)   Date: 02/21/90
CAS#:   12674-11-2
Office: OTTRS/ODW
Previously Verified: No
Previous Discussion Dates: None.

Outstanding Issues: None

1.   Documentation:

     Adequate. However, ODW questioned the usefulness of RfDs for
     individual Arochlors as their occurrence in the environment is
     as a mixture. OTTRS noted Region 5 requested an RfD for this
     specific Arochlor (Attachment #5). OHEA noted the lack of a
     multigeneration study as a gap in the data base.

2.   Study:

     Appropriate. HERL pointed out that there was a lower dose
     group which was not included in the study description. OTTRS
     was asked to obtain these data (Allen work).

3.   Uncertainty Factor: Appropriate

4.   Modifying Factor: None

5.   Calculation: Correct

6.   Confidence Statement: Not discussed

7.   Are the old issues resolved: None

8.   Outstanding issues:

     The work group is considering whether RfDs should be written
     for individual Arochlors.

9.   Additional work:

     OTTRS was asked to include the Allen work, check the CRAVE
     meeting notes for the Arochlor discussion, and talk to the
     regions about Arochlor mixtures in the field. ODW was asked
     to write-up their position and show comparable toxicity data
     for other Arochlors for the work group to consider.

3

10. New Status: The RfD for Arochlor 1016 is

ON IRIS: NOT ON IRIS:

_____   No change to IRIS (IR)   _____   Verified (V)
_____   Pending change to IRIS (RE)   ___X___   Under Review (UR)
_____   Withdraw and new RfD   _____   Not Verified (NV)
                    Verified (WV)
_____   Withdraw and Still
                    Under Review (WR)

New Verification Date: _____

4

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2
Preparation Date -- 01/20/90

__I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Reduced birth weight	NOEL: 0.25 ppm diet (approx. 0.01 mg/kg bw/day)	100	1	1E-4 mg/kg/day
Monkey Reproductive Bioassay				
Barsotti and van Miller, 1984	LOAEL: 1.0 ppm diet (approx. 0.04 mg/kg bw/day)			

*Conversion Factors: Dietary concentrations were converted to mg/kg bw/day
dose rates by assuming that the pregnant monkeys consumed 4.2% of their body
weight per day on average.

__I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Barsotti, D.A. and J.P. van Miller. 1984. Accumulation of a commercial
polychlorinated biphenyl mixture (Aroclor 1016) in adult rhesus monkeys and
their nursing infants. Toxicology. 30: 31-44.

In animals, Aroclor 1016 given in the diet to 24 female rhesus monkeys
for 87 plus or minus 9 weeks at 0, 0.25 or 1.0 ppm (8 animals/group) evoked
no similarities of clinical, gross or reproductive parameters in the
animals. The animals were bred, conceived and experienced uncomplicated
pregnancies. The birth weight of infants in the control, 0.25 and 1.0 ppm
groups were 518 plus or minus 64, 491 plus or minus 24, and 422 plus or minus
29 g, respectively. The high-dose birth weight was significantly smaller
than control (p<0.01). No significant differences between experimental and
control groups were detected in neonatal head circumference or crown to rump
measurements. Both experimental groups showed consistent weight gains, but
at weanling, infant weights from the high-dose group remained lower (although
not statistically so). This study demonstrated a NOEL of 0.25 ppm and a
LOAEL of 1.0 ppm.

In humans, Yamashita (1977) reported four cases of infants born to
mothers who had Yusho during pregnancy. The amount of PCB-contaminated oil
consumed during pregnancy was approximately 1.1-10.5 L. Maternal symptoms
included acneform eruptions, follicular accentuation; dark brown pigmentation
on the skin, mucous membranes and nails; and hypersecretion of the meibomian
gland. Three of the four infants, including one full-term (40 weeks
gestation), one premature (36 weeks gestation), and one 2 weeks later than
term (42 weeks gestation), were small-for-gestational age (both weight and

height). Other clinical features among the four infants included dark brown pigmentation on the skin and mucous membranes, ging' l hyperplasia, eruption of teeth at birth, spotted calcification on the parieto-occipital skull and the large or wide fontanels and sagittal suture, facial edema and exophthalmic eyes.

Kuratsune et al. (1969) summarized four studies of 10 live and 3 stillborn births from February 15 to January 31, 1968, to 11 females with Yusho during pregnancy and 2 wives of males with Yusho during the female's pregnancy. The amount of Kanechlor-contaminated oil consumed during pregnancy was 0.3-2.6 L (Yamaguchi et al., 1971). Of 10 live and 2 stillborn births, 9 had unusually grayish, dark-brown stained skin, 5 had similar pigmentation of the gingiva and nails and most had increased eye discharge (Yamaguchi et al., 1971; Taki et al., 1969; Funatsu et al., 1971). Of the 13 infants, 12 were described as smaller than the national Japanese standards and 4 as small-for-dates babies (Yamaguchi et al., 1971; Taki et al., 1969).

A study of individuals who consumed moderate quantities of PCB-contaminated lake fish indicated that PCBs crossed the placenta. PCB exposure, as measured by both contaminated fish consumption and cord serum PCB levels, predicted lower birth weight and smaller head circumference of infants born to these mothers (Fein et al., 1984).

High PCB serum levels were found in some women who had recent or former missed abortions with mean PCB serum levels of 103.04, 82.00 and 20.69 ppb for recent missed abortions, former missed abortions and control groups, respectively (Bercovici et al., 1983). Some women with premature delivery had mean PCB serum levels of 128 ppb in the premature delivery group vs. 26.5 ppb in the control group (Wasserman et al., 1982). The higher PCB serum levels were associated with increased incomplete abortions (Bercovici et al., 1983) and premature deliveries (Wasserman et al., 1982), but a definitive causal relationship cannot be established, as only small numbers of women were examined (up to 17 symptomatic; up to 10 asymptomatic), and some of these women had high serum levels of some organochlorine insecticides (DDT isomers and their metabolites, lindane, dieldrin, heptachlor epoxide).

The effects discussed in these human studies are almost certainly evoked by different PCBs (indeed the Japanese studies were reporting on the Yusho incident which was also caused in part by exposure to polychlorinated dibenzofurans). However, a consistent finding among these human studies is a reduced birth weight. While not definitive in their own right, these human studies indirectly support the Agency's choice of the critical effect from the Barsotti and van Miller (1984) study, used as the basis of the RfD.

____I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF = 100. This represents a 10-fold factor to account for sensitive individuals and another factor of 10 to account for uncertainties in both animal to human (about 3-fold) and subchronic to chronic (about 3-fold). A full 10-fold factor for animal to human is not considered necessary because of the similarity in metabolism between monkeys and humans (V. supra), and

the general similarity between these two species. A full 10-fold factor for subchronic to chronic is not considered necessary because of interim duration of the exposure in the critical study.

MF = 1.

___I.A.4. ADDITIONAL COMMENTS (ORAL RfD)

Commercial PCB mixtures vary in PCB isomer and congener composition, and impurities. In general, PCB mixtures produce low to moderate acute toxicity in mammalian species, but produce pronounced toxicity after short-term, subchronic and chronic exposures. In addition, as reported for other halogenated aromatic hydrocarbons, PCBs exhibit significant interspecies variability in toxicity. In considering the health effects of PCBs in animals, it is important to consider the isomer and congener composition of the PCBs, potential impurities, the length of exposure and the species under investigation. The data presented in this IRIS file refers specifically to Aroclor 1016 and should not be used for other PCBs without proper analysis.

Aroclor 1016 given in the diet to pastel mink of both sexes for 8 months at 0 (24 females and 6 males) or 20 ppm (12 females and 3 males) induced emaciation characterized by an almost complete absence of body fat in 3/12 females that died. The 3/24 females that died in the control group did not exhibit this extreme condition (Bleavins et al., 1980). Aroclor also reduced reproductive performance as only 4/9 females mated produced kits (versus 16/21 in control). Aroclor 1016 given in the diet to 8 female and 2 male mink at 2 ppm for 10 months evoked no significant differences in body weight gain, hemoglobin or packed cell volume. Nor were effects seen on reproductive parameters, kit growth, or adult or kit mortality (Aulerich and Ringer, 1977). These two studies indicate a range in the possible experimental threshold for the reproductive and developmental toxicity of Aroclor 1016 in mink of between 2 and 20 ppm of diet. The dose-severity slope appears to be steep, however, and neither study tested sufficient animals in order to draw definitive conclusions.

Aroclor 1016 given in the diet to about 15 BALB/CJ male mice for 6 weeks at 167 ppm increased the mortalities caused by S. typhosa endotoxin and P. berghei, but failed to demonstrate histopathological changes in lung, thymus, mesenteric lymph nodes or spleen. Histopathological examination of the liver revealed hepatocytic hyperplasia (Loose et al., 1978). Aroclor 1016 given in the diet to an unreported number of C57Bl/6 male mice for 3 weeks at 167 ppm elicited a greater graft versus host response from splenic cells from treated mice when injected into neonates. This indicates the Aroclor 1016 may activate donor lymphocytes (Silkworth and Loose, 1978). These latter two studies indicate that immunological effects are occurring in mice at dietary concentrations of about 8- or 170-fold higher than concentrations that evoke reproductive and developmental effects in mink and monkeys, respectively.

In the past 60 years, large numbers of workers have been exposed to PCBs in the manufacture or use of PCB-containing products; however, evaluation of any health effects is complicated by exposure to other chemicals. Generally speaking, symptoms associated with PCB exposure do not correlate with duration and intensity of exposure in the workplace (U.S. EPA, 1988). For

example, it appears that individual susceptibility to chloracne is more important than duration and extent of PCB exposure. This data indicate that a reduction in the UF for subchronic to chronic exposure may be appropriate.

Data exists on the in vitro hepatic metabolism and in vivo metabolic clearance of 2,2',3,3',6,6'-hexa-CB and 4,4'-di-CB in humans, monkeys, dogs and rats (Schnellmann et al., 1985). For each PCB, the Vmax values for metabolism in the monkey, dog and rat are consistent with the respective metabolic clearance values generated from in vivo studies. Thus, the kinetic constants for PCB metabolism obtained from the dog, monkey and rat hepatic microsomal preparations were good predictors of in vivo metabolism and clearance for these PCBs.

In investigations directed at determining which species most accurately predicts the metabolism and disposition of PCBs in humans, the in vitro metabolism of 2,2',3,3',6,6'-hexa-CB and 4,4'-di-CB was also investigated in human liver microsomes (Schnellmann et al., 1983, 1984). Available data suggest that the human metabolism of PCBs would most closely resemble that of the monkey and rat, but not the dog. For example, the in vitro apparent K_m (um) and Vmax (pmoles/nmoles P-450/min) are comparable between humans and monkeys with values for the Kms of 2,2',3,3',6,6'-hexa-CB and 4,4'-di-CB of 8.8 and 0.43 in humans and 5.2 and 0.92 in monkeys, respectively. Values for Vmax for these two PCBs were 19 and 4.4 in humans as compared to 14 and 4.3 in monkeys, respectively. In vivo data on the relative persistence of specific PCBs in humans are also consistent with the above in vitro results on the metabolism of PCBs.

These metabolism studies collectively indicate that the monkey is comparable to humans for these two PCBs and that in vitro results are consistent with in vivo results. The use of a monkey study as a basis of the RfD may thus reduce the need for a full (traditional) UF of 10 for experimental animal to man extrapolation.

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study: Medium
Data Base: Medium
RfD: Medium

The critical study rates a medium confidence. It was well conducted in a sensitive animal species that closely resembles man in many respects. A sensitive measure of PCB toxicity was monitored. The data base rates a medium to low confidence. Although specific data on Aroclor 1016 are not extensive, the critical effect is consistent with those of other PCBs and the available human toxicity data are not inconsistent with the animal findings. Medium to low confidence in the RfD follows.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

U.S. EPA. 1988. Drinking Water Criteria Document for Polychlorinated
Biphenyls (PCBs). Environmental Criteria and Assessment Office, Cincinnati,
OH. ECAO-CIN-414, April.

Agency RfD Work Group Review: 02/ /90

Verification Date:

___I.A.7. EPA CONTACTS (ORAL RfD)

Michael L. Dourson / ORD -- (513)569-7531 / FTS 684-7531

Krishan Khanna / ODW -- (202)382-7588 / FTS 382-7588

_VI. REFERENCES

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DRAFT

SUBJECT: RfD/RfC Work Group Meeting--March 24, 25 and 26, 1992

TO: RfD/RfC Work Group

FROM: Annie M. Jarabek
Office of Health and Environmental Assessment
Research Triangle Park, NC

Michael L. Dourson
Office of Health and Environmental Assessment
Cincinnati, OH

IN ATTENDANCE WERE:

NAME	OFFICE	PHONE
	March 24, 1992	
K. Bailey	OW	260-5535
M. Barron	OSW	260-6977
R. Benson	Region VIII	330-1543
J. Cicmanec	OHEA/STA	684-7481
S. Chou	ATSDR	236-6037
M. Copley	OPP/HED	
S. Dapson	OPP/HED	305-7661
B. Davis	OSWER	260-9493
M. Dourson	OHEA/STA	684-7533
G. Foureman	OHEA/HPA	629-1183
E. Francis	OTTRS/ORD	260-7891
G. Ghali	OPP/HED	365-7490
L. Hall	HERL/ORD	629-2774
R. Hill	OPPTS	260-2897
L. Ingerman	SRC	(315) 426-3405
A. Jarabek	OHEA/HPA	629-4847
T. McMahon	OPP/HED	
B. Means	OERR	260-2201
B. Mintz	OW/OST	260-9569
M. Morris	OW/OST	260-0312
E. Ohanian	OW/OST	260-7571
Y. Patel	OW	260-5849
W. Phang	OPP/HED	
K. Poirier	OHEA/CMA	684-7462
J. Risher	Region IV	257-1586
S. Rotenberg	Region III	597-2842
S. Segal	Clement	(703) 934-3768
P. White	OHEA/EAG	260-2589
R. Whiting	OPP/HED	365-5473

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Outstanding Issues: OTTRS to include Allen work and discuss with the regions which aroclor mixtures are found in the environment. OST was asked to write-up their position and show comparable toxicity data for other aroclors.

1.   Documentation:

     Sufficient. OHEA/STA distributed a handout (Attachment #11) on the distribution of PCB congeners in different aroclor formulations.

2.   Rationale:

     A series of studies in the same group of monkeys (Barsotti and van Miller 1984; Levin et al. 1988; Schantz et al. 1989, 1991) were chosen as co-critical studies. In these studies, the monkeys were exposed to a commercial mixture of aroclor 1016 (containing no chlorinated dibenzofurans) prenatally and throughout lactation until weaning at 4 months. Behavioral testing began at 14 months of age (with choice discrimination-reversal tests) and again at 4-6 years of age with a delayed spatial alternation test. Reduced birth weights and learning deficits in offspring that occurred at a LOAEL of 18.4 mg/kg for 21.8 months (0.028 mg/kg/day) (no NOAEL established) were identified as the critical effects. There was a significant difference between the high-dose group (0.028 mg/kg/day) and the controls in the first behavioral test conducted at 14 months, and the two treated groups were significantly different from each other but not from control in the second behavioral test conducted at 4-6 years.

3. Study:

OW noted that they had reservations regarding the analytical characterization of aroclors, their concerns included (1) the presence of trace contaminants cannot be ruled out and (2) the arochlor mixtures present in the environment are different than those used experimentally. The Work Group viewed OW concerns as risk characterization issues rather than issues regarding dose-response.

Hyperpigmentation was observed in the offspring of animals exposed to both 0.007 mg/kg/day and 0.028 mg/kg/day and this effect was not considered adverse; however, OHEA/STA expressed concern with how to handle this effect. The Work Group agreed with OHEA/STA that the hyperpigmentation at the low-dose level was of concern, but not considered to be adverse, especially since the effect was reversible.

Region VIII suggested that the 0.007 mg/kg/day dose level be called out as a NOAEL and that the critical studies should be considered developmental studies rather than reproductive studies.

OHEA/HPA suggested that the low-dose level may be a LOAEL for learning deficits. The Work Group was uncomfortable in interpreting the learning data and suggested that HERL/NTD review the effects.

4. Uncertainty Factor:

The uncertainty factor will be influenced by the NOAEL/LOAEL designation and the determination if the critical studies are developmental studies.

5. Modifying Factor: Not discussed

6. Calculation: Not discussed

7. Confidence Statement: Not discussed

8. Are the old issues resolved: None

9. Outstanding issues:

OHEA/STA to ask HERL/NTD to review the critical studies to determine if the low-dose level is a LOAEL.

10. Additional work:

1) OW was asked to provide OHEA/STA with a statement of their concerns about the characterization of the aroclors. OHEA/STA was asked to incorporate this statement into the additional comments section.

2) OW requested that the discussions for each of the critical studies be better delineated.

3) As discussed in #3.

11. New Status: The RfD for aroclor 1016 is

ON IRIS: NOT ON IRIS:

_____ No change to IRIS (IR)   _____ Verified (V)
_____ Pending change to IRIS (RE)   __X__ Under Review (UR)
_____ Withdraw and new RfD   _____ Not Verifiable (NV)
             Verified (WV)
_____ Withdraw and Still
             Under Review (WR)

New Verification Date: _____

12

_I. *CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

_I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Aroclor 1016
CASRN -- 12674-11-2
Preparation Date -- Pending

_I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Reduced birth weights; Learning deficits in offspring	NOAEL: None	1000	1	3E-5 mg/kg/day
	LOAEL: 18.4 mg/kg for 21.8 months (0.028 mg/kg/day)			
Monkey Reproductive Bioassay				

Barsotti and van Miller,
1984; Levin et al., 1988;
Schantz et al., 1989, 1991

*Conversion Factors: Dosage corresponds to a reported total average intake of 4.52 mg/kg bw during an average exposure period of 21.8 months (Schantz et al., 1989, 1991). Aroclor 1016 was administered as 0.25 ppm in the diet.

_I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Barsotti, D.A. and J.P. van Miller. 1984. Accumulation of a commercial polychlorinated biphenyl mixture (Aroclor 1016) in adult rhesus monkeys and their nursing infants. Toxicology. 30: 31-44.

Levin, E.D., S.L. Schantz and R.E Bowman. 1988. Delayed spatial alternation deficits resulting from perinatal PCB exposure in monkeys. Arch. Toxicol. 62: 267-273.

Schantz, S.L., E.D. Levin, R.E. Bowman et al. 1989. Effects of perinatal PCB exposure on discrimination-reversal learning in monkeys. Neurotoxicol. Teratol. 11: 243-250.

Schantz, S.L., E.D. Levin and R.E. Bowman. 1991. Long-term neurobehavioral effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys. Environ. Toxicol. Chem. 10: 747-756.

These are a series of reports that evaluated perinatal toxicity and long-term neurobehavioral effects of Aroclor 1016 in the same groups of infant monkeys. Aroclor 1016 was administered to groups of 8 adult female rhesus monkeys (body weight not reported) via diet in concentrations of 0, 0.25 or 1.0 ppm for 21.8+/-2.2 months. The Aroclor 1016 was a commercial mixture found to be devoid of chlorinated dibenzofurans (Barsotti and van Miller, 1984).

Exposure began 7 months prior to breeding (6 control females and 7 exposed females per dosage were bred to unexposed males) and continued until offspring were weaned at age 4 months. Based on a reported total Aroclor intake of 4.52+/-0.56 and 18.41+/-3.64 mg/kg over the 21.8-month exposure period (Schantz et al., 1989, 1991), the low and high dosages are estimated to be 0.007 and 0.028 mg/kg/day, respectively. No exposure-related effects on maternal food intake, general appearance, hematology (complete blood counts), serum chemistry (SGPT, lipid and cholesterol analyses) or number of breedings were observed (Barsotti and van Miller, 1984). All monkeys had uncomplicated pregnancies, carried their infants to term and delivered viable offspring. No teratologic examinations were performed. Mean birth weights of the infants in the control, 0.007 and 0.028 mg/kg/day dosage groups were 512+/-64 g, 491+/-24 g and 422+/-29 g, respectively (Barsotti and van Miller, 1984). The decrease in birth weight in the high dosage group was significantly ($p < 0.01$) lower than the age-matched controls. No significant differences between treatment and control groups were detected in neonatal head circumference or crown-to-rump measurements. Both exposure groups showed consistent weight gains, but infant weights in the high dosage group were still lower (864+/-97 g) at weaning although not significantly different from the controls (896+/-90 g). It was not reported whether the hyperpigmentation was present at birth or developed during nursing, which may be likely due to concentration of Aroclor 1016 in breast milk and consequent higher than maternal mg/kg/day dose. No exposure-related hematologic effects were observed in the infants during the nursing period (Barsotti and van Miller, 1984). One of the offspring in the high dosage group went into shock and died on the day following weaning for unknown reasons (Schantz et al., 1989, 1991).

Behavioral testing of the infant monkeys was first performed at age 14 months and no overt signs of PCB toxicity were observed (Schantz et al., 1989, 1991). Two-choice discrimination-reversal learning was assessed using simple left-right spatial position, color and shape discrimination problems, with and without irrelevant color and shape cues. One of the offspring in the low dosage group stopped responding early in testing for an unknown reason and could not be induced to resume; therefore, test results were obtained using 6, 7 and 6 infants in the control, low and high dosage groups, respectively. The offspring in the high dosage (0.028 mg/kg/day) group were significantly ($p < 0.05$) impaired in their ability to learn the spatial position discrimination problem (i.e., acheive 9 correct choices in 10 trials), requiring more than 2.5 times as many trials as their age-matched controls. There were no significant learning differences between these groups on this problem during overtraining (ability to achieve greater than or equal to 90% correct choices in two consecutive daily sessions) or position reversals. The only other exposure-related effect was significantly ($p < 0.05$) facilitated learning ability on the shape discrimination problem at 0.028 mg/kg/day.

Performance on delayed spatial alternation (a spatial learning and memory task) was assessed in the offspring monkeys at age 4-6 years (Levin et al., 1988; Schantz et al., 1991). The two Aroclor-exposed groups were not significantly different ($p < 0.05$) from controls in test performance. However, the exposed groups did significantly ($p < 0.05$) differ from each other. The difference between the two exposed groups was due to a combination of facilitated performance at the low dosage (0.007 mg/kg/day) and impaired performance at the high dosage (0.028 mg/kg/day). Although these data are

insufficient for establishing an exposure-effect relation due to the lack of difference between exposed and control groups, the investigators suggested that the performance deficit at 0.028 mg/kg/day may have been exposure-related. The investigators noticed that a paradoxical biphasic effect occurred on the same test when comparing low dose and high dose infants. This same effect has been observed for lead-exposed monkeys. Impaired performance at higher doses may be due to a more pronounced reduction of attention that detracted from the cues critical for performing the task itself.

As summarized above, monkeys that ingested 0.007 or 0.028 mg/kg/day dosages of Aroclor 1016 for approximately 22 months terminating during lactation showed no evidence of maternal toxicity. Effects occurred in the infants of these monkeys which consisted of hairline hyperpigmentation at greater than or equal to 0.007 mg/kg/day, and decreased birth weight and possible neurological impairment at 0.028 mg/kg/day. Although incompletely described and reversible following cessation of lactation exposure, evidence from other studies of PCBs indicates that the increased skin pigmentation was potentially adverse. In particular, hyperpigmention is part of the spectrum of mildly adverse dermal effects characteristic of PCBs and related compounds. Dermal lesions including skin irritation, chloracne and increased pigmention of skin and nails have been observed in humans occupationally exposed to Aroclor 1016 and other Aroclor formulations by both inhalation and dermal routes (Fischbein et al., 1979, 1982, 1985; Ouw et al., 1976; Smith et al., 1982). Insufficient data are available to determine possible contributions of Aroclor 1016 alone, direct skin exposure and contaminants in these occupational studies. Chloracne and other dermal lesions, including dark brown hyperpigmentation of the gingival and buccal mucosa, lips, conjunctivae and nails, are prominant manifestions in people who consumed heated rice oil contaminated with Kanechlor PCBs in Japan (Yusho incident) and Taiwan (Yu-Cheng incident) (Kuratsune, 1989; Kashimoto and Miyata, 1989; Rogan, 1989). Additionally, babies born live or stillborn to mothers who had Yusho and Yu-Cheng exposure during pregnancy had similar hyperpigmentation and other dermal lesions. Effects of Yusho and Yu-Cheng exposure cannot be attributed specifically to PCBs due to relatively high concentrations of polychlorinated dibenzofurans (PCDFs), which are generally thought to be the primary causal agent (Kuratsune, 1989; Kashimoto and Miyata, 1989). These studies do demonstrate, however, sensitivity of humans to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-like toxicity, which is relevant to Aroclor 1016 assessment because Aroclor 1016 contains congeners structurally similar to TCDD and dibenzofuran. Dermal effects similar to those associated with human occupational and Yusho/Yu-Cheng PCB exposure are well documented in monkeys following subchronic oral exposure to Aroclors 1248 or 1254 (Allen and Norback, 1976; Allen et al., 1973, 1974; Barsotti et al., 1976; Becker et al., 1979; Tryphonas et al., 1986a,b). Existing information therefore strongly suggests that chronic direct exposure to Aroclor 1016 is likely to have caused dermal effects in monkeys more severe than the reversible hyperpigmentation resulting from transplacental/transmammary exposure alone. Additionally, the oral studies of Aroclors 1248 and 1254 in monkeys indicate that exposures sufficient to cause dermal lesions are also likely to cause nondermal effects such as developmental and hepatic toxicity. Based on the learning deficits described above and the reduced birth weights of prenatally-exposed monkeys, the 0.028 mg/kg/day dosage is a LOAEL in monkeys.

Decreased birth weight has also been reported in infants born to women who were occupationally exposed to Aroclor 1016 and other Aroclor formulations (Taylor et al., 1984, 1989), ingested PCB-contaminated fish (Fein et al., 1984a,b) and ingested heated Kanechlor PCBs during the Yusho and Yu-Cheng incidents (Rogan, 1989; Yamashita, 1977). Due to uncertainities regarding actual sources of PCB exposure, contaminants and other confounding factors and study limitations, the decreases in human birth weight cannot be attributed to PCBs, particularly specific PCB mixtures. However, due to the consistency with which the effect has been observed, the human data indirectly support the Aroclor 1016-induced decreased birth weight in monkeys. The results of the neurobehavioral tests in the monkey offspring at 14 months and 4-6 years of age indicate adverse learning deficits at the 0.028 mg/kg/day maternal dosage. Evaluation of these data is complicated by possible inconsistencies in the outcome of both the discrimination-reversal learning tests (learning was impaired and facilitated on different problems) and the delayed spatial alternation test (performance significantly differed between the two exposed groups, but not between either control group and the control). However, there is evidence suggesting that deficits in discrimination-reversal learning and delayed spatial alternation are related to decreased brain dopamine (Schantz et al., 1991), which has been observed in monkeys orally exposed to Aroclor 1016 (Seegal et al., 1990, 1991). Behavioral dysfunctions, including deficits in visual recognition and short-term memory, also have been observed in infants of human mothers who consumed fish contaminated with unknown PCB mixtures (Fein et al., 1984a,b; Jacobsen et al., 1985, 1990; Gladen et al., 1988).

___I.A.3 UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF=1000. This represents a 10-fold factor to account for sensitive individuals, and factors of 3-fold for each of the following: extrapolation from animal to human, extrapolation from LOAEL to NOAEL, extrapolation from subchronic to chronic and incomplete data base. A full 10-fold factor for interspecies extrapolation is not considered necessary because of similarities in toxic responses and metabolism of PCBs between monkeys and humans and the general physiologic similarity between these species. A full 10-fold factor for estimating a NOAEL from a LOAEL is not considered necessary because the LOAEL was only mildly adverse as indicated by lack of maternal toxicity. A <10-fold factor is used to extrapolate from subchronic to chronic duration because it is uncertain if the learning deficits in the infant monkeys is developmental-related (i.e., should be considered equivalent to chronic exposure) or due to nondevelopmental toxicity. A <10-fold factor is used to reflect limitations of the data base (lack of a 2-generation reproduction study, teratology study and adequate toxicity studies in two species).

MF=1.

Male macaque monkeys (number not reported, age 3-7 years, 5-9 kg inital body weight) were administered Aroclor 1016 dissolved in corn oil on bread in dosages of 0, 0.8, 1.6 or 3.2 mg/kg/day for 20 weeks (Seegal et al., 1991). There were no overt signs of intoxication or exposure-related effects on body weight gain. Neurochemical analyses of various regions of the brain were performed following termination of exposure. Dose-related decreased concentrations of dopamine were observed in the caudate nucleus, putamen, substantia nigra and hypothalamus, but not in the globus pathidus or hippocampus. There were no exposure-related changes in concentrations of norepinephrine, epinephrine or serotonin. Other neurologic endpoints were not evaluated in this study.

Subchronic oral studies of Aroclor 1016 have been performed in species other than monkeys. As summarized below, these species were tested at dosages higher than the 0.007 and 0.028 mg/kg/day dosages fed to monkeys in the principal studies.

Groups of 10 female Sprague-Dawley rats (age not reported, body weight approximately 225-250 g at start) were fed 0, 1, 5 or 50 ppm Aroclor 1016 (purity and lot number not reported) in the diet for 5 months (Byrne et al., 1988). The Aroclor was dissolved in acetone which was evaporated from the diet prior to feeding. Using a rat food consumption factor of 0.05 kg food/kg bw, the dosages are estimated to be 0.05, 0.25 and 2.5 mg/kg/day. Serum levels of adrenal cortex hormones were evaluated 4 times throughout the treatment period. Adrenal dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHS) levels were significantly ($p<0.05$) reduced at 0.05 mg/kg/day and higher doses after approximately 100 days of exposure. Serum corticosterone (the principal glucocorticoid in rats), adrenal weight, adrenal histology and nonadrenal endpoints other than food consumption were not evaluated. Food consumption did not significantly differ between and among control and treatment groups. Because insufficient information is available to determine whether the decreases in circulating adrenal hormones were physiologically significant, it is uncertain whether the dosages are NOAELs or LOAELs for Aroclor 1016 in rats.

Male Balb/c mice (age not reported, 18-20 g body weight at start) were fed Aroclor 1016 (purity and lot number not reported) mixed in diet at concentrations of 0 or 5 ppm for 3 or 6 weeks (Loose et al., 1978). Using a mouse food consumption factor of 0.13 kg food/kg bw, the dosage is estimated to be 0.65 mg/kg/day. Sensitivity to Salmonella typhosa endotoxin (15 mice per endotoxin dose) and resistance to infection by Plasmodium berghei (malaria parasitemia; number of mice not reported) were evaluated. Sensitivity to the endotoxin was significantly ($p<0.05$) increased after 3 weeks of exposure as indicated by endotoxin LD50 values of 152 and 844 ug in the Aroclor-exposed and control groups, respectively. Sensitivity to the endotoxin after 6 weeks of Aroclor exposure was not evaluated. There were no significant ($p<0.05$) effects of Aroclor exposure for 3 or 6 weeks on malaria lethality as indicated by postinoculation survival time. No other endpoints were evaluated in this study. Splenic cells from C57Bl/6 male mice (18-20 g body weight at start, number and age not reported) exposed to 167 ppm (21.71 mg/kg/day) dietary Aroclor 1016 for 3 weeks elicited a greater graft-versus-host reaction than

controls when injected into neonates (Silkworth and Loose, 1978). Based on the decreased resistance to infection leading to death, 0.65 mg Aroclor 1016/kg/day is a LOAEL for immunotoxicity for subchronic exposure in male mice.

Aulerich and Ringer (1977) performed a breeding study in which groups of 8 female and 2 male adult pastel mink (body weight not reported) were fed diets containing 0 or 2 ppm Aroclor 1016 (purity and lot number not reported) for 39 weeks or until the kits were 4 weeks of age. The Aroclor was dissolved in acetone which was evaporated from the diet prior to feeding. Using assumed values of 150 g/day for food consumption and 0.8 kg for body weight for female mink (Bleavins et al., 1980), the estimated dosage of Aroclor 1016 is 0.4 mg/kg/day. Monthly determinations showed no statistically significant (p<0.05) differences between the control and treated mink in body weight gain, hemoglobin, and hematocrit. Additionally, tabulated data showed no treatment-related effects on female survival, numbers of females mated, number of females that gave birth, number of kits born alive or dead, number of births per female, average birth weight or number of kits alive at 4 weeks. Additional salient information regarding the design or results of this study were not reported. The evidence for lack of treatment-related effects on body weight, hematology, reproduction and survival suggests that 0.4 mg/kg/day is a NOEL for Aroclor 1016 in mink.

Groups of adult Pastel mink (body weight not reported) were fed diet containing 0 ppm (24 females and 6 males) or 20 ppm (12 females and 3 males) Aroclor 1016 (purity and lot number not reported) during a 247-day breeding study (Bleavins et al., 1980). The Aroclor was dissolved in acetone which was evaporated from the diet prior to feeding. Using assumed values of 150 g/day for food consumption and 0.8 kg for body weight for female mink reported by the investigators, the estimated dosage of Aroclor 1016 is 3.8 mg/kg/day. There were no deaths in the exposed or control males. Mortality was higher in the exposed females [25% (3/12) compared to 12.5% (3/24) in controls], but no clear difference in survival time was observed. Necropsies for gross abnormalities were performed on all control and treated mink that died; these showed effects only in the treated mink consisting of emaciation characterized by an almost complete absence of body fat. Histologic examinations were not performed. The incidence of mated females giving birth was reduced in the exposed group [44.4% (4/9) compared to 76.2% (16/21) in controls], but average gestation length, live births and birth weight did not significantly differ (p>0.05) between exposed and control groups. Body weight at age 4 weeks, average number of infants per lactating female and infant biomass (average body weight gain through age four weeks x average number of infants raised per lactating female) were significantly (p<0.05) reduced in the exposed group. Mortality during the first 4 weeks of life was increased in the exposed group [56.0% (14/25) compared to 24.1% (19/79) in controls]. The investigators noted that the adverse effects on reproduction do not appear to be due to an effect on spermatogenesis since PCB-treated male mink have had acceptable levels of reproduction when mated to untreated females in other studies. The evidence for impaired reproduction and increased maternal and postnatal mortality suggests that 3.8 mg Aroclor 1016/kg/day is an FEL in mink. Although the FEL from this study and NOEL of 0.4 mg/kg/day from Aulerich and Ringer (1977) suggest that the dose-severity slope for Aroclor 1016 in mink is steep, neither study tested sufficient numbers of animals or dosage levels to draw definitive conclusions.

Human data directly useful for risk assessment of Aroclor 1016 are not
available. Studies of the general population who presumably were exposed to
PCBs by consumption of contaminated food, particularly neurobehavioral
evaluations of infants exposed in utero and/or through lactation, have been
reported, but the original PCB mixtures, exposure levels and other details of
exposure are not known (Kreiss et al., 1981; Humphrey, 1983; Fein et al.,
1984a,b; Jacobson et al., 1984a, 1985, 1990a,b; Rogan et al., 1986; Gladen et
al., 1988). Most of the information on health effects of PCB mixtures in
humans is available from studies of occupational exposure. Some of these
studies examined workers who had some occupational exposure to Aroclor 1016,
but sequential or concurrent exposure to other Aroclor mixtures nearly always
occurred, exposure involved dermal as well as inhalation routes (relative
contribution by each route not known), and monitoring data are lacking or
inadequate (Fischbein et al., 1979, 1982, 1985; Fischbein, 1985; Warshaw et
al., 1979; Smith et al., 1982; Lawton et al., 1985).

Information specifically on the oral absorption of Aroclor 1016 is not
available, but studies of individual congeners and PCB mixtures of higher
chlorine content in animals indicate, in general, that PCBs are readily and
extensively absorbed. These studies have found oral absorption efficiency on
the order of 75 to >90% in rats, mice, monkeys and ferrets (Albro and
Fishbein, 1972; Allen et al., 1974; Tanabe et al., 1981; Bleavins et al.,
1984; Clevenger et al., 1989). A study of a 54% chlorine PCB mixture provides
direct evidence of absorption of PCBs in humans after oral exposure (Buhler et
al., 1988), and indirect evidence of oral absorption of PCBs by humans is
available from studies of ingestion of contaminated fish by the general
population (Schwartz et al., 1983; Kuwabara et al., 1979). There are no
quantitative data regarding inhalation absorption of PCBs in humans but
studies of workers exposed suggest that PCBs are well absorbed by the
inhalation and dermal routes (Maroni et al., 1981a,b; Smith et al., 1982;
Wolff, 1985). PCBs distribute preferentially to adipose tissue and
concentrate in human breast milk due to its high fat content (Jacobson et al.,
1984b; Ando et al., 1985).

The metabolism of PCBs following oral and parenteral administration in
animals has been extensively studied and reviewed, but studies in animals
following inhalation or dermal exposure are lacking (Sundstrom and Hutzinger,
1976; Safe, 1980; Sipes and Schnellmann, 1987). Information on metabolism of
PCBs in humans is limited to occupationally exposed individuals whose intake
is derived mainly from inhalation and dermal exposure (Jensen and Sundstrom,
1974; Wolff et al., 1982; Schnellmann et al., 1983; Safe et al., 1985; Fait et
al., 1989). In general, metabolism of PCBs depends on the number and position
of the chlorine atoms on the phenyl ring of the constituent congeners (i.e.,
congener profile of the PCB mixture) and animal species. Although only
limited data are available on metabolism of PCBs following inhalation
exposure, there is no reason to suspect that PCBs are metabolized differently
by this route.

Data exist on the in vitro hepatic metabolism and in vivo metabolic
clearance of 2,2',3,3',6,6'-hexachlorobiphenyl and 4,4'-dichlorobiphenyl
congeners in humans, monkeys, dogs and rats (Schnellmann et al., 1985). Both
of these congeners are present in Aroclor 1016, but the hexachlorbiphenyl is
only a minor constituent. For each congener, the Vmax values for metabolism
in the monkey, dog and rat are consistent with the respective metabolic

clearance values found in vivo. Thus, the kinetic constants for PCB metabolism obtained from the dog, monkey and rat hepatic microsomal preparations were good predictors of in vivo metabolism and clearance for these congeners. In investigations directed at determining which species most accurately predicts the metabolism and dispostion of PCBs in humans, the in vitro metabolism of these congeners was also studied using human liver microsomes (Schnellmann et al., 1983, 1984). Available data suggest that metabolism of PCBs in humans would most closely resemble that of the monkey and rat. For example, the in vitro apparent Km and Vmax are comparable between humans and monkeys. These studies show consistency between the in vitro and in vivo findings and collectively indicate that metabolism of the two congeners is similar in monkeys and humans.

__I.A.5. CONFIDENCE IN THE ORAL RfD

Study: Medium
Data Base: Medium
RfD: Medium

The critical study (i.e., series of studies on the same animals) rates a medium confidence. This was well conducted in a sensitive animal species that closely resembles man in many respects, and evaluated sensitive endpoints of PCB toxicity in maternal animals and offspring. The data base rates medium confidence based on limited toxicity and reproductive data in different species. Although specific data on Aroclor 1016 are not extensive, the critical effect is consistent with those of other PCBs and the available human toxicity data are consitent with the animal findings. Medium confidence in the RfD follows.

__I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

U.S. EPA. 1980. Ambient Water Quality Criteria Document for Polychlorinated Biphenyls. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC. EPA-440/5-80/068. NTIS PB81-117798/AS.

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Agency RfD Work Group Review:

Verification Date:

__I.A.7. EPA CONTACTS (ORAL RfD).

John L. Cicmanec / ORD — (513)569-7481 / FTS 684-7481

/   — ( )   -   / FTS

_VI.   REFERENCES

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
CINCINNATI. OHIO 45268

SUBJECT: RfD/RfC Work Group Meeting--June 23, 24, and 25, 1992

TO: RfD/RfC Work Group

FROM: Daniel Guth   7-2 8-93
(Acting Co-Chair for Annie M. Jarabek)
Office of Health and Environmental Assessment
Research Triangle Park, NC

Michael L. Dourson   7 23·93
Office of Health and Environmental Assessment
Cincinnati, OH

Kenneth A. Poirier
(Acting Co-Chair for M. Dourson)   7·23·93
Office of Health and Environmental Assessment
Cincinnati, OH

Chemical Name: Arochlor 1016 (RfD)   Date: 06/23/92
CAS#: 12674-11-2
Office: OHEA/STA
Previous Decision: Under Review
Previous Discussion Dates: 02/21/90, 03/25/92

Outstanding Issues: The Work Group requested OHEA/STA to ask
HERL to review learning deficit data from critical studies.

1.   Documentation: Adequate.

2.   Rationale:

     A series of developmental toxicity studies in the same group
     of monkeys (Barsotti and van Miller 1984; Levin et al. 1988;
     Schantz et al. 1989, 1991) were chosen as co-critical
     studies. In these studies, the monkeys were exposed to a
     commercial mixture of Arochlor 1016 (containing no
     chlorinated dibenzofurans) prenatally and throughout
     lactation until weaning at 4 months. Behavioral testing
     began at 14 months of age (two choice discrimination-
     reversal test) and again at 4-6 years of age (delayed
     spatial alternation test). Reduced birth weight that
     occurred at a LOAEL of 18.4 mg/kg for 21.8 months (0.030
     mg/kg/day) (NOAEL = 4.6 mg/kg for 21.8 months [0.008
     mg/kg/day]) was identified as the critical effect. OHEA/STA
     conducted additional statistical analyses with raw data
     obtained from the investigators. Based on the results of
     these analyses, gestation length, sex, and sire ID were
     ruled out as causes of the significant decrease in birth
     weight observed in the high-dose animals. At the Work
     Group's request, HERL/NTD evaluated the neurobehavioral
     data, and concluded that because the dosed groups did not
     differ significantly from control (even though they differed
     significantly from each other), neurotoxicity should not be
     considered a critical effect. Hyperpigmentation was
     observed in the offspring of animals exposed to both 0.008
     mg/kg/day and 0.030 mg/kg/day, but this effect was not
     considered adverse. OPPT questioned the appropriateness of
     the dose calculation; the doses were calculated for the
     entire dosing period (including the lactation period) but
     the critical effect occurred during gestation and at birth.
     OPPT suggested that the doses be calculated based on food
     intake and body weight during pregnancy only. OHEA/STA
     responded that these data were not available, but agreed to
     add some text that more accurate doses could not be
     calculated. The Work Group concurred with the choice of
     critical study and effect.

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3. Study:

    OHEA/STA stated that R. MacPhail (HERL/NTD) evaluated the
    Schantz et al. (1989, 1991) and Levin et al. (1988) studies.
    Dr. MacPhail concluded that since the neurological effects
    observed in the arochlor 1016 exposed monkeys did not differ
    from the controls, neurotoxicity should not be highlighted
    as an effect.

    OHEA/STA obtained exact birth weight data from the
    investigators. Gestational length, sex and sire were
    factored out in the statistical analysis of the data
    (Attachment #5). A significant reduction in birth weight
    was observed in the high dose group. The Work Group agreed
    with the selection of the principal studies, critical effect
    and NOAEL/LOAEL designation.

4. Uncertainty Factor:

    OHEA/RDT suggested that an additional uncertainty factor of
    3 be included for the lack of male reproductive toxicity
    data and a multigeneration reproduction study. Region VIII
    commented that an uncertainty factor of 3 for use of a
    subchronic study was not needed because the principal study
    is a developmental toxicity study. OHEA/HHAG responded that
    there was a potential for longer term effects due to storage
    in adipose tissue. OPPT suggested decreasing the
    interspecies variability uncertainty factor to 3 because a
    sensitive species is being used. OHEA/CMA commented that
    they were uncomfortable with an overall uncertainty factor
    of 100, with medium confidence in the study, data base and
    RfD. After extended discussion, the Work Group agreed with
    an overall uncertainty factor of 100; 3H, 3A, 3S, and 3DB.
    The Work Group asked that the discussions of the uncertainty
    factors for intra- and inter-species extrapolation, and lack
    of lifetime data and data base deficiencies be combined.

5. Modifying Factor: None

6. Calculation:

    OPPT commented that the doses for the principal studies
    should not be calculated for the duration of the entire
    dosing period but rather up until parturition. OHEA/STA
    responded that the data were not available to calculate
    doses until parturition. The Work Group requested that
    OHEA/STA include a statement to this effect.

7. Confidence Statement:

   OHEA/RDT requested that the data base gap (lack of male reproduction and multigeneration reproduction studies) be called out. The Work Group agreed with medium confidence in the study, data base and RfD.

8. Are the old issues resolved: Yes.

9. Outstanding issues: None

10. Additional work:

   1) OHEA/STA was asked to revise the statement on page 2 that arochlor 1016 in breast milk was higher than the maternal mg/kg/day dose.

   2) OST was asked to include a discussion of risk characterization for arochlors. OST agreed.

   3) The Work Group requested that Dr. MacPhail's comments on the neurological effects be included in the IRIS file.

   4) OHEA/STA was asked to rewrite the uncertainty factor text to call out 4 partial areas of uncertainty.

11. New Status: The RfD of 8 E-5 mg/kg/day for arochlor 1016 is

ON IRIS: NOT ON IRIS:

_____ No change to IRIS (IR)   __X__ Verified (V)
_____ Pending change to IRIS (RE)   _____ Under Review (UR)
_____ Withdraw and new RfD   _____ Not Verifiable (NV)
         Verified (WV)
_____ Withdraw and Still
         Under Review (WR)

New Verification Date: __06/23/92__

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
CINCINNATI. OHIO 45268

DRAFT

SUBJECT: RfD/RfC Work Group Meeting--September 22, 23 and 24, 1992

TO: RfD/RfC Work Group

FROM: Annie M. Jarabek
Office of Health and Environmental Assessment
Research Triangle Park, NC

Daniel Guth
(Acting co-chair for A. Jarabek on September 22 and 23)
Office of Health and Environmental Assessment
Research Triangle Park, NC

Michael L. Dourson
Office of Health and Environmental Assessment
Cincinnati, OH

1

GENERAL INTEREST

1. Daniel Guth (ECAO/HPA) served as acting co-chair for Annie Jarabek on September 22 and 23.

2. OHEA/STA requested that methyl mercury RfD be withdrawn from the agenda.

3. OHEA/HPA requested that the d-limonene RfD be withdrawn from the agenda.

4. Due to time constraints, the RfC for dichlorvos was not discussed at the meeting.

GENERAL DISCUSSION

Arochlor 1016 RfD – John Cicmanec (OHEA/STA)

At the September 22 meeting, John Cicmanec discussed correspondence on Arochlor 1016 from General Electric. General Electric raised a number of issues regarding the proposed RfD summary sheet (verified by the RfD/RfC Work Group) in an August 20, 1992 discussion with John Skinner and Bill Farland. These points were re-emphasized in a follow-up letter from Stephen Ramsey to Hank Habicht (dated September 3, 1992). As noted by John Cicmanec, these issues were discussed in detail at the previous two Work Group meetings. The newly revised summary sheet provides clarification to the General Electric issues by specific additions to the text pertaining to description of the principal study, uncertainty factor, and confidence statement. The Work Group was asked to submit comments on the revised summary sheet to John.

IRIS RfD Background Document –Bob Benson (Region VIII)

At the September 24 meeting, Bob Benson presented a draft of the revised IRIS RfD Background Document (Attachment 2) and requested comments from the Work Group. Several members felt that the discussion on biological significance (Section 1.3.1.1) was confusing and suggested using a different example. It was also suggested that this discussion belongs in Section 1.3.1.2.2.
Bob requested that Work Group members provide minor and editorial comments to him in writing. These comments will be incorporated and another draft circulated.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
CINCINNATI. OHIO 45268

November 16, 1992

SUBJECT: Review Notes of the Special October 15th Teleconference Meeting for the
RfD/RfC Work Group

FROM: Annie M. Jarabek
Co-Chair, RfD/RfC Work Group
Office of Health and Environmental Assessment

Kenneth A. Poirier, Ph.D.
Co-Chair, RfD/RfC Work Group
Office of Health and Environmental Assessment

TO: RfD/RfC Work Group Members

ALDICARB:

OPP described the changes in the summary sheet that were made in response to the discussion held on September 23rd at the previous Work Group meeting. The RfD is still based on the NOAEL described in the Rhone-Poulonc (1992) acute human study that identified the critical effect as sweating. The rationale for choosing this effect and the short duration of exposure is detailed in 1.A.3. of the summary sheet on page 6. The proposed UF of 10 was questioned. Region 1 thought that the agreed upon UF at the previous meeting was 30, not 10. OHEA-Cin responded that a UF of 10 was an open question pending additional explanation as provided above. Region 8 questioned why headaches were not considered as the critical effect at the lowest dose of exposure in the Rhone-Poulonc study. OPP reiterated the comments from the previous meeting. Headaches are consistent with carbamate exposure, however, a joint OPPT/OW/OHEA expert panel discounted headaches as a possible effect of treatment in this particular exposure group because the headaches appeared toward the end of the 6 hour observation time, rather than soon after exposure as expected from other data on carbamates. OPP agreed to add this additional information to the summary sheet. OHEA-HQ introduced the comments provided in a memo by Elaine Francis (OHEA) and questioned the choice of an UF of 10 especially in light of how uncertainty factors have historically been assigned. OHEA-RTP raised a concern on the subjectivity of the measurements for the appearance and degree of sweating reported in the Rhone Poulonc (1992) study. OSW and Region 8 questioned the lack of adequate documentation for the absence of long-term human sequelae. However, it was pointed out that the complete data base supports the endpoint of sweating as the critical effect based on the consequences of cholinesterase inhibition. OPP also responded that it is more difficult to justify an additional UF given what is known specifically for aldicarb and the carbamates in general. Although several members of the Work Group expressed some reservation for using an UF_H of 10, after extended discussion this value had the consensus of the Work Group. OHEA commented that UF as proposed is consistent with what has been historically used in other files. OPP stated that this reflects the data known for human poisoning episodes.

Additional discussion was centered around the confidence statements. Given the lack of knowledge of long-term human effects, the confidence in the data base was deemed to be medium. Additional, study confidence in the text was suggested as being medium to low, given the issues of subjectivity of reporting effects, duration of exposure and small subject number in the Rhone-Poulonc (1992) study. There was no change suggested for the confidence of the RfD.

The RfD for Aldicarb was verified at 1E-3 mg/kg-day on 10/15/92.

AROCHLOR 1016:

The primary contact, John Cicmanec, was unable to attend the special teleconference due to previously scheduled travel. Mike Dourson filled in for John Cicmanec. The changes to the text that were made in response to General Electric's comments to both Hank Habicht and Erich Bretthauer were highlighted along with a

more detailed memo from John Cicmanec to the Work Group regarding these points. It was agreed that although there are some deficiencies in the Arochlor 1016 primate studies, OHEA-HQ pointed out that they are minor in light of the fact that behavioral testing in primates are much more reliable than any other non-human species and that non-human primates represent the best choice for determining critical effects. OHEA-HQ also pointed out that there were no problems with using these studies relative to reproductive or developmental toxicity endpoints.

The Work Group was in unanimous agreement that the primate studies of Barsotti meet the criteria of developing an RfD for Arochlor 1016. Unfortunately, due to the length of time of the preceding discussion, insufficient time was available in the assigned teleconference window to complete discussion of revisions to the Arochlor 1016 summary sheet. A final decision was, therefore, deferred to the November meeting.

The RfD for Arochlor 1016 is verified pending approval of the summary sheet by the RfD/RfC Work Group.

RfD/RfC Work Group Members/Mailing List:

Larry Anderson (TS-796)
Monica Barron (OS-331)
Robert Beliles (RD-689)
Robert Benson (Region 8)
Nancy Chiu (WH-586)
John Cicmanec (ECAO-Cin)
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RfD file for Aldicarb
RfD file for Arochlor 1016

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Environmental Criteria and Assessment Office (MD-52)
Research Triangle Park, North Carolina 27711

DATE: July 6, 1993

SUBJECT: RfD/RfC Work Group Meeting Notes from November 4-5, 1992

FROM: Gary L. Foureman
            Acting Co-Chair, RfD/RfC Work Group
            Office of Health and Environmental Assessment
            Environmental Criteria and Assessment Office/RTP (MD-52)

            Kenneth A. Poirier
            Co-Chair, RfD/RfC Work Group
            Office of Health and Environmental Assessment
            Environmental Criteria and Assessment Office/CIN (MS-114)

Chemical Name: Arochlor 1016   (RfD)   Date: 11/04/92
CAS#: 12674-11-2
Office: OHEA/STA
Previous Decision: V
Previous Discussion Dates: RfD: 02/21/90, 03/25/92, 06/23/92, 09/24/92, 10/15/92;
RfC: None; CRAVE: None

Outstanding Issues: Address submitted comments in the text.

1.   Documentation:

Adequate. Verified in June. Material received by ORD management following the June verification warranted a reexamination of the RfD summary sheet by the primary contact. This resulted in additional clarification and documentation of the uncertainty factor text, revision/expansion of the confidence statement with an emphasis on the close parallel between changes seen in the Rhesus monkey and humans and a clarification that the principal study is a reproductive toxicity study. OHEA/HPA questioned the significance of the Taylor et al. (1984, 1989) studies discussed in the Principal and Supporting Studies section. OHEA/STA responded that these studies provided positive results for developmental effects in humans and were most supportive of the critical effect seen in monkeys. OHEA/HPA requested that the discussion of the Yusho and Yu-Cheng incidents be deemphasized and moved to the Additional Comments section because the effects seen in these incidents were due primarily to PCDFs and not PCBs. Region VIII requested that since the Barsotti Ph.D. dissertation was cited in the text of the summary sheet it should be included in the references. OST requested that a statement be added to the effect that environmental exposures to the PCBs will be different than experimental exposure to commercial mixtures (i.e., Arochlor 1016). OHEA/HHAG mentioned that it is also important to point out that these compounds (i.e., the PCBs) are known to have adverse ecological effects and that the RfD is protective only for human health.

2.   Rationale:

A series of developmental toxicity studies in the same group of monkeys (Barsotti and van Miller 1984; Levin et al. 1988; Schantz et al. 1989, 1991) were chosen as co-principal studies. In these studies, monkeys were exposed to a commercial mixture of Arochlor 1016 (containing no chlorinated dibenzofurans) prenatally and throughout lactation until weaning at 4 months. Behavioral testing began at 14 months of age (two choice discrimination-reversal test) and again at 4-6 years of age (delayed spatial alternation test). Reduced birth weight occurred at a LOAEL of 18.4 mg/kg for 21.8 months (0.03 mg/kg/day) (NOAEL = 4.6 mg/kg for 21.8 months [0.008 mg/kg/day]) and was identified as the critical effect. OHEA/STA conducted additional statistical analyses with raw data obtained from the investigators. Based on the results of these analyses, gestation length, sex, and sire ID were ruled out as causes of the significant decrease in birth weight observed in the high-dose animals. Hyperpigmentation was observed in the offspring of

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animals exposed to both 0.008 mg/kg/day and 0.030 mg/kg/day, but this effect was not considered adverse. The Work Group concurred with the choice of principal study and critical effect.

3.   Study:

Region VIII requested that a more complete discussion of the feed contamination problems in this study be incorporated and should include the fact that contamination with other PCBs occurred in all groups and that the concentration of the contaminants was lower than those at which a decrease in birth weight would likely be seen. OHEA/HPA pointed out that there is a discrepancy between the Schantz et al. papers and the Barsotti paper with regard to the birth weight of the infants, and that this discrepancy should be pointed out. Region VIII noted that the OHEA/STA statistical analyses included data from fathers that were treated with Arochlor 1248 and that these data should be excluded from the analyses. The Work Group noted that exclusion of these data should have no impact on the results. (A statement should be added to the text that 1-2 males in each group received Arochlor 1248 instead of Arochlor 1016, but that further analysis showed that this did not affect the results.

4.   Uncertainty Factor:

In the revised summary sheet presented to the Work Group, OHEA/STA changed the uncertainty factors from four factors of 3 to a factor of 10 for sensitive individuals, a factor of 3 for interspecies extrapolation, and a factor of 3 for database deficiencies. OHEA/RDTB noted that according to the developmental toxicity risk assessment guidelines, an additional uncertainty factor should be incorporated to account for less than chronic duration exposure when calculating an RfD. This uncertainty factor is obviated only when deriving an RfD_{DT}. Region III questioned why an uncertainty factor of 3 was used to extrapolate from monkeys to humans. OHEA/HHAG responded that previous work with Rhesus monkeys (e.g., Tilson's work) provides good support that the monkey is a better predictor of toxicity in humans than any rodent species and that a full uncertainty factor of 10 is thus not necessary. OHEA/CMA stated that four uncertainty factors of 3 each for UF[H], UF[A], UF[S], and UF[D] is a more tenable position. The critical points for database deficiencies include: the principal study has not been repeated, and in general, there is a lack of multigeneraltional reproductive toxicity studies and specifically, a lack of information on effects on reproductive function in males that were exposed in utero . OHEA/HPA questioned why UF_H = 3. OHEA/STA responded that the effect was seen in what is presumed to be the most sensitive subset of the population (i.e., the fetus of non-human primate). The Work Group agreed to a total uncertainty factor of 100 comprised of four factors of 3 each.

5.   Modifying Factor: None

6. Calculation: Not discussed.

7. Confidence Statement: _

The confidence statement was rewritten as described in #1 above. OHEA/HHAG questioned why the confidence in the study was not high because of the way it is written, and suggested that the weaknesses of the database and study be more clearly delineated to justify the medium confidence rating. OHEA/HPA requested that there be a separate discussion of the study and database confidence because as it is now written, the confidence statement focuses primarily on the study. The Work Group agreed to medium confidence in the study, data base, and RfD.

8. Are the old issues resolved:

Yes. The Work Group felt that the changes incorporated into the summary sheet adequately addressed the points submitted to ORD management.

9. Outstanding issues: None

10. Additional work:

1) The uncertainty factor text has to be rewritten to reflect four factors of 3.

2) The confidence statement has to be rewritten to separately discuss the database and to more clearly point out the deficiencies in the study and the database.

3) The discussion of the Yusho and Yu-Cheng incidents needs to be deemphasized and moved into the Additional Comments section.

4) Discussion has to be added to the Additional Comments section regarding the fact that environmental exposures will not be the same as commercial mixtures and that PCBs have adverse ecological effects.

5) The feed contamination problem in the principal study has to be more fully discussed.

6) The discrepancy in the birth weights between the Schantz et al. papers and the Barsotti papers needs to be mentioned.

7) A statement should be added to the discussion of the principal studies that 1-2 males in each group received Arochlor 1248 instead of Arochlor 1016, but that this did not affect the results.

__I.A.   REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Chemical -- Aroclor 1016
CASRN -- 12674-11-2   THIS IS THE LATEST VERSION ! !
On-line: Pending

___I.A.1.   ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Reduced birth weights	NOAEL: 4.6 mg/kg for 21.8 months (0.008 mg/kg/da)	100		1.8E-5 mg/kg/d
	LOAEL: 18.4 mg/kg for 21.8 months (0.03 mg/kg/day)			
Monkey Reproductive Bioassay				
Barsotti and van Miller, 1984; Levin et al., 1988; Schantz et al., 1989, 1991				

*Conversion Factors:Dosage corresponds to a reported total average intake of
4.52 mg/kg bw during an average exposure period of 21.8 months (Schantz et
al., 1989, 1991). Arochlor 1016 was administered as 0.25 ppm in the diet.

___I.A.2.   PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Barsotti, D.A. and J.P. van Miller. 1984. Accumulation of a commercial
polychlorinated biphenyl mixture (Arochlor 1016) in adult rhesus monkeys and
their nursing infants. Toxicology. 30: 31-44.

Levin, E.D., S.L. Schantz and R.E Bowman. 1988. Delayed spatial alternation
deficits resulting from perinatal PCB exposure in monkeys. Arch. Toxicol.
62: 267-273.

Schantz, S.L., E.D. Levin, R.E. Bowman et al. 1989. Effects of perinatal PCB
exposure on discrimination-reversal learning in monkeys. Neurotoxicol.
Teratol. 11: 243-250.

Schantz, S.L., E.D. Levin and R.E. Bowman. 1991. Long-term neurobehavioral
effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys.
Environ. Toxicol. Chem. 10: 747-756.

     These are a series of reports that evaluated perinatal toxicity and
long-term neurobehavioral effects of Arochlor 1016 in the same groups of
infant monkeys. Arochlor 1016 was administered to groups of 8 adult female
rhesus monkeys (body weight not reported) via diet in concentrations of 0,
0.25 or 1.0 ppm for 21.8+/-2.2 months. The Arochlor 1016 is a commercial
mixture devoid of chlorinated dibenzofurans (Barsotti and van Miller, 1984).
Exposure began 7 months prior to breeding (6 control females and 7 exposed

females per dose were bred to unexposed males) and continued until offspring were weaned at age 4 months. Based on a reported total Arochlor intake of 4.52+/-0.56 and 18.41+/-3.64 mg/kg over the 21.8-month exposure period (Schantz et al., 1989, 1991), the low and high doses are estimated to be 0.008 and 0.03 mg/kg/day, respectively. No exposure-related effects on maternal food intake, general appearance, hematology, serum chemistry (SGPT, lipid and cholesterol analyses) or number of breedings were observed (Barsotti and van Miller, 1984). All monkeys had uncomplicated pregnancies, carried their infants to term and delivered viable offspring. Teratologic examinations were not performed. Mean birth weights of the infants in the control, 0.008 and 0.03 mg/kg/day dose groups were 512+/-64 g, 491+/-24 g and 422+/-29 g, respectively (Barsotti and van Miller, 1984). The decrease in birth weight in the high dose group was significantly (p<0.01) lower than controls. Further statistical analysis of the infant birth weight data indicated that gestation length did not significantly affect birth weight and the distribution of male and female infants in the various dose groups could not account for the difference in birth weights among the dose groups. No significant differences between treatment and control groups were detected in neonatal head circumference or crown-to-rump measurements. Both exposure groups showed consistent weight gains, but infant weights in the high dose group were still lower (864+/-97 g) at weaning although not significantly different from the controls (896+/-90 g). Hyperpigmentation was present at birth in the low and high dose infants but did not persist once dosing was stopped. This clinical change was determined not to be a critical adverse effect. The concentration of Arochlor 1016 in breast milk was higher than the maternal mg/kg/day dose. No exposure-related hematologic effects were observed in the infants during the nursing period (Barsotti and van Miller, 1984). One of the offspring in the high dose group went into shock and died on the day following weaning for unknown reasons (Schantz et al., 1989, 1991).

Behavioral testing of the infant monkeys was first performed at age 14 months and no overt signs of PCB toxicity were observed (Schantz et al., 1989, 1991). Two-choice discrimination-reversal learning was assessed using simple left-right spatial position, color and shape discrimination problems, with and without irrelevant color and shape cues. One of the offspring in the low dosage group stopped responding early in testing for an unknown reason and could not be induced to resume; therefore, test results were obtained using 6, 7 and 6 infants in the control, low and high dosage groups, respectively. The offspring in the high dosage (0.03 mg/kg/day) group were significantly (p<0.05) impaired in their ability to learn the spatial position discrimination problem (i.e., achieved 9 correct choices in 10 trials), requiring more than 2.5 times as many trials as their age-matched controls. There were no significant learning differences between these groups on this problem during overtraining (ability to achieve greater than or equal to 90% correct choices in two consecutive daily sessions) or position reversals. The only other exposure-related effect was significantly facilitated learning ability (p<0.05) on the shape discrimination problem at 0.03 mg/kg/day.

Performance on delayed spatial alternation (a spatial learning and memory task) was assessed in the offspring monkeys at age 4-6 years (Levin et al., 1988; Schantz et al., 1991). The two Arochlor-exposed groups were not significantly different from controls (p<0.05) in test performance. However, the exposed groups did significantly (p<0.05) differ from each other. The

difference between the two exposed groups was due to a combination of facilitated performance at the low dose (0.008 mg/kg/day) and impaired performance at the high dose (0.03 mg/kg/day). Although these data are insufficient for establishing an exposure-effect relation due to the lack of difference between exposed and control groups, the investigators suggested that the performance deficit at 0.03 mg/kg/day may have been exposure-related. The investigators noticed that a paradoxical biphasic effect occurred on the same test when comparing low-dose and high-dose infants. This same effect has been observed for lead-exposed monkeys. Impaired performance at higher doses may be due to a more pronounced reduction of attention that detracted from the cues critical for performing the task itself.

As summarized above, adult monkeys that ingested 0.008 or 0.03 mg/kg/day dosages of Arochlor 1016 for approximately 22 months, terminating during lactation, showed no evidence of maternal toxicity. Effects occurred in the infants of these monkeys consisting of hairline hyperpigmentation at greater than or equal to 0.008 mg/kg/day, and decreased birth weight and possible neurological impairment at 0.03 mg/kg/day. Dermal lesions including skin irritation, chloracne and increased pigmentation of skin and nails have been observed in humans occupationally exposed to Arochlor 1016 and other Arochlor formulations by both inhalation and dermal routes (Fischbein et al., 1979, 1982, 1985; Ouw et al., 1976; Smith et al., 1982). Insufficient data are available to determine possible contributions of Arochlor 1016 alone, direct skin exposure and contaminants in these occupational studies. Chloracne and other dermal lesions, including dark brown hyperpigmentation of the gingival and buccal mucosa, lips, conjunctivae and nails, are prominent manifestations in people who consumed heated rice oil contaminated with Kanechlor PCBs in Japan (Yusho incident) and Taiwan (Yu-Cheng incident) (Kuratsune, 1989; Kashimoto and Miyata, 1986; Rogan, 1989). Additionally, babies born live or stillborn to mothers who had Yusho and Yu-Cheng exposure during pregnancy had similar hyperpigmentation and other dermal lesions. Effects of Yusho and Yu-Cheng exposure cannot be attributed specifically to PCBs due to relatively high concentrations of polychlorinated dibenzofurans (PCDFs), which are generally thought to be the primary causal agent (Kuratsune, 1989; Kashimoto and Miyata, 1989). These studies demonstrate human toxicity similar to that seen with the nonhuman primate studies. Sensitivity of humans to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is relevant to Arochlor 1016 assessment because Arochlor 1016 contains congeners structurally similar to TCDD and dibenzofuran. Dermal effects similar to those associated with human occupational and Yusho/Yu-Cheng PCB exposure are well documented in monkeys following subchronic oral exposure to Aroclors 1248 or 1254 (Allen and Norback, 1976; Allen et al., 1973, 1974; Barsotti et al., 1976; Becker et al., 1979; Tryphonas et al., 1986a,b). Based on the reduced birth weights of prenatally-exposed monkeys, the 0.03 mg/kg/day dose is a LOAEL in monkeys.

Decreased birth weight has also been reported in infants born to women who were occupationally exposed to Arochlor 1016 and other Arochlor formulations (Taylor et al., 1984, 1989), ingested PCB-contaminated fish (Fein et al., 1984a,b) and ingested heated Kanechlor PCBs during the Yusho and Yu-Cheng incidents (Rogan, 1989; Yamashita, 1977). Due to uncertainties regarding actual sources of PCB exposure, and other confounding factors and study limitations, the decreases in human birth weight cannot be solely attributed to PCBs, particularly specific PCB mixtures. However, due to the consistency with which the effect has been observed, the human data is

consistent with the Arochlor 1016-induced decreased birth weight in monkeys. The results of the neurobehavioral tests in the monkey offspring at 14 months and 4-6 years of age indicate adverse learning deficits at the 0.03 mg/kg/day maternal dose. Evaluation of these data is complicated by possible inconsistencies in the outcome of both the discrimination-reversal learning tests (learning was impaired and facilitated on different problems) and the delayed spatial alternation test (performance significantly differed between the two exposed groups, but not between either control group and the control). However, there is evidence suggesting that deficits in discrimination-reversal learning and delayed spatial alternation are related to decreased brain dopamine (Schantz et al., 1991), which has been observed in monkeys orally exposed to Arochlor 1016 (Seegal et al., 1990, 1991). Behavioral dysfunctions, including deficits in visual recognition and short-term memory, also have been observed in infants of human mothers who consumed fish contaminated with unknown PCB mixtures (Fein et al., 1984a,b; Jacobsen et al., 1985, 1990; Gladen et al., 1988).

___I.A.3.   UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF = A 10-fold factor is applied to account for sensitive individuals. A factor of 3 is applied for extrapolation from rhesus monkeys to human. A full 10-fold factor for interspecies extrapolation is not considered necessary because of similarities in toxic responses and metabolism of PCBs between monkeys and humans and the general physiologic similarity between these species. The rhesus monkey data is predictive of other changes noted in human studies such as chloracne, hepatic changes, and effects on reproductive function. An additional factor of 3 is applied because of limitations in the data base. Despite the extensive amount of animal laboratory data and human epidemiologic information regarding PCBs, the issue of male reproductive effects is not directly addressed and two-generation reproductive studies are not available.

MF = 1.

___I.A.4.   ADDITIONAL STUDIES / COMMENTS (ORAL RfD)

Male macaque monkeys (Macaca nemistrina age 3-7 years, 5-9 kg initial body weight) were administered Arochlor 1016 dissolved in corn oil on bread in doses of 0, 0.8, 1.6 or 3.2 mg/kg/day for 20 weeks (Seegal et al., 1991). There were no overt signs of intoxication or exposure-related effects on body weight gain. Neurochemical analyses of various regions of the brain were performed following termination of exposure. Dose-related decreased concentrations of dopamine were observed in the caudate nucleus, putamen, substantia nigra and hypothalamus, but not in the globus pallidus or hippocampus. There were no exposure-related changes in concentrations of norepinephrine, epinephrine or serotonin. Other neurologic endpoints were not evaluated.

Subchronic oral studies of Arochlor 1016 have been performed in species other than monkeys. As summarized below, these species were tested at doses higher than the 0.008 and 0.03 mg/kg/day doses fed to monkeys in the principal studies.

Groups of 10 female Sprague-Dawley rats (age not reported, body weight approximately 225-250 g at start) were fed 0, 1, 5 or 50 ppm Arochlor 1016 (purity and lot number not reported) in the diet for 5 months (Byrne et al., 1988). The Arochlor was dissolved in acetone that was evaporated from the diet prior to feeding. Using a rat food consumption factor of 0.05 kg food/kg bw, the doses are estimated to be 0.05, 0.25 and 2.5 mg/kg/day. Serum levels of adrenal cortical hormones were evaluated 4 times throughout the treatment period. Adrenal dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHS) levels were significantly ($p<0.05$) reduced at 0.05 mg/kg/day and higher doses after approximately 100 days of exposure. Serum corticosterone (the principal glucocorticoid in rats), adrenal weight, adrenal histology and nonadrenal endpoints other than food consumption were not evaluated. Food consumption did not significantly differ between and among control and treatment groups. Because insufficient information is available to determine whether the decreases in circulating adrenal hormones were physiologically significant, it is uncertain whether the doses are NOAELs or LOAELs for Arochlor 1016 in rats.

Male Balb/c mice (age not reported, 18-20 g body weight at start) were fed Arochlor 1016 mixed in diet at concentrations of 0 or 5 ppm for 3 or 6 weeks (Loose et al., 1978). Using a mouse food consumption factor of 0.13 kg food/kg bw, the dosage is estimated to be 0.65 mg/kg/day. Sensitivity to Salmonella typhosa endotoxin (15 mice per endotoxin dose) and resistance to infection by Plasmodium berghei (malaria parasitemia; number of mice not reported) were evaluated. Sensitivity to the endotoxin was significantly ($p<0.05$) increased after 3 weeks of exposure as indicated by endotoxin LD50 values of 152 and 844 ug in the Arochlor-exposed and control groups, respectively. Sensitivity to the endotoxin after 6 weeks of Arochlor exposure was not evaluated. There were no significant ($p<0.05$) effects of Arochlor exposure for 3 or 6 weeks on malaria lethality as indicated by post inoculation survival time. No other endpoints were evaluated in this study. When injected into neonates, splenic cells from C57Bl/6 male mice exposed to 167 ppm (21.71 mg/kg/day) dietary Arochlor 1016 for 3 weeks elicited a greater graft-versus-host reaction than controls (Silkworth and Loose, 1978). Based on the decreased resistance to infection leading to death, 0.65 mg Arochlor 1016/kg/day is a LOAEL for immunotoxicity for subchronic exposure in male mice.

Aulerich and Ringer (1977) performed a breeding study in which groups of 8 female and 2 male adult pastel mink were fed diets containing 0 or 2 ppm Arochlor 1016 for 39 weeks or until the kits were 4 weeks of age. The Arochlor was dissolved in acetone which was evaporated from the diet prior to feeding. Using assumed values of 150 g/day for food consumption and 0.8 kg for body weight for female mink (Bleavins et al., 1980), the estimated dosage of Arochlor 1016 is 0.4 mg/kg/day. Monthly determinations showed no statistically significant differences ($p<0.05$) between the control and treated mink in body weight gain, hemoglobin, and hematocrit. Additionally, tabulated data showed no treatment-related effects on female survival, numbers of females mated, number of females that gave birth, number of kits born alive or dead, number of births per female, average birth weight or number of kits alive at 4 weeks. The evidence for lack of treatment-related effects on body weight, hematology, reproduction and survival suggests that 0.4 mg/kg/day is a NOEL for Arochlor 1016 in mink.

Groups of adult Pastel mink (body weight not reported) were fed a diet containing 0 ppm (24 females and 6 males) or 20 ppm (12 females and 3 males) Arochlor 1016 during a 247-day breeding study (Bleavins et al., 1980). The Arochlor was dissolved in acetone which was evaporated from the diet prior to feeding. Using assumed values of 150 g/day for food consumption and 0.8 kg for body weight for female mink reported by the investigators, the estimated dosage of Arochlor 1016 is 3.8 mg/kg/day. There were no deaths in the exposed or control males. Mortality was higher in the exposed females [25% (3/12) compared to 12.5% (3/24) in controls], but no clear difference in survival time was observed. Necropsies for gross abnormalities were performed on all control and treated mink that died; these showed effects only in the treated mink consisting of emaciation characterized by an almost complete absence of body fat. Histologic examinations were not performed. The incidence of mated females giving birth was reduced in the exposed group [44.4% (4/9) compared to 76.2% (16/21) in controls], but average gestation length, live births and birth weight did not significantly differ (p>0.05) between exposed and control groups. Body weight at age 4 weeks, average number of infants per lactating female and infant biomass (average body weight gain through age four weeks x average number of infants raised per lactating female) were significantly (p<0.05) reduced in the exposed group. Mortality during the first 4 weeks of life was increased in the exposed group [56.0% (14/25) compared to 24.1% (19/79) in controls]. The investigators noted that the adverse effects on reproduction do not appear to be due to an effect on spermatogenesis since PCB-treated male mink have had acceptable levels of reproduction when mated to untreated females in other studies. The evidence for impaired reproduction and increased maternal and postnatal mortality suggests that 3.8 mg Arochlor 1016/kg/day is an FEL in mink. Although the FEL from this study and NOEL of 0.4 mg/kg/day from Aulerich and Ringer (1977) suggest that the dose-severity slope for Arochlor 1016 in mink is steep, neither study tested sufficient numbers of animals or dosage levels to allow definitive conclusions to be drawn.

The human data available useful for risk assessment of Arochlor 1016 are useful only in a qualitative manner. Studies of the general population who were exposed to PCBs by consumption of contaminated food, particularly neurobehavioral evaluations of infants exposed in utero and/or through lactation, have been reported, but the original PCB mixtures, exposure levels and other details of exposure are not known (Kreiss et al., 1981; Humphrey, 1983; Fein et al., 1984a,b; Jacobson et al., 1984a, 1985, 1990a,b; Rogan et al., 1986; Gladen et al., 1988). Most of the information on health effects of PCB mixtures in humans is available from studies of occupational exposure. Some of these studies examined workers who had some occupational exposure to Arochlor 1016, but in these studies concurrent exposure to other Arochlor mixtures nearly always occurred, exposure involved dermal as well as inhalation routes (relative contribution by each route was not known), and monitoring data were lacking or inadequate (Fischbein et al., 1979, 1982, 1985; Fischbein, 1985; Warshaw et al., 1979; Smith et al., 1982; Lawton et al., 1985).

Information specifically on the oral absorption of Arochlor 1016 is not available, but studies of individual congeners and PCB mixtures of higher chlorine content in animals indicate, in general, that PCBs are readily and extensively absorbed. These studies have found oral absorption efficiency on the order of 75 to >90% in rats, mice, monkeys and ferrets (Albro and

Fishbein, 1972; Allen et al., 1974; Tanabe et al., 1981; Bleavins et al., 1984; Clevenger et al., 1989). A study of a PCB mixture containing 54 % chlorine provides direct evidence of absorption of PCBs in humans after oral exposure (Buhler et al., 1988), and indirect evidence of oral absorption of PCBs by humans is available from studies of ingestion of contaminated fish by the general population (Schwartz et al., 1983; Kuwabara et al., 1979). There are no quantitative data regarding inhalation absorption of PCBs in humans but studies of exposed workers suggest that PCBs are well absorbed by the inhalation and dermal routes (Maroni et al., 1981a,b; Smith et al., 1982; Wolff, 1985). PCBs distribute preferentially to adipose tissue and concentrate in human breast milk due to its high fat content (Jacobson et al., 1984b; Ando et al., 1985).

The metabolism of PCBs following oral and parenteral administration in animals has been extensively studied and reviewed, but studies in animals following inhalation or dermal exposure are lacking (Sundstrom and Hutzinger, 1976; Safe, 1980; Sipes and Schnellmann, 1987). Information on metabolism of PCBs in humans is limited to occupationally exposed individuals whose intake is derived mainly from inhalation and dermal exposure (Jensen and Sundstrom, 1974; Wolff et al., 1982; Schnellmann et al., 1983; Safe et al., 1985; Fait et al., 1989). In general, metabolism of PCBs depends on the number and position of the chlorine atoms on the phenyl rings of the constituent congeners (i.e., congener profile of the PCB mixture) and animal species. Although only limited data are available on metabolism of PCBs following inhalation exposure, there is no reason to suspect that PCBs are metabolized differently by this route.

Data exist on the in vitro hepatic metabolism and in vivo metabolic clearance of 2,2',3,3',6,6'-hexachlorobiphenyl and 4,4'-dichlorobiphenyl congeners in humans, monkeys, dogs and rats (Schnellmann et al., 1985). Both of these congeners are present in Arochlor 1016, but the hexachlorobiphenyl is only a minor constituent. For each congener, the Vmax values for metabolism in the monkey, dog and rat are consistent with the respective metabolic clearance values found in vivo. Thus, the kinetic constants for PCB metabolism obtained from the dog, monkey and rat hepatic microsomal preparations were good predictors of in vivo metabolism and clearance for these congeners. In investigations directed at determining which species most accurately predicts the metabolism and disposition of PCBs in humans, the in vitro metabolism of these congeners was also studied using human liver microsomes (Schnellmann et al., 1983, 1984). Available data suggest that metabolism of PCBs in humans most closely resemble that of the monkey and rat. For example, the in vitro apparent Km and Vmax for humans and monkeys are comparable. These studies show consistency between the in vitro and in vivo findings and collectively indicate that metabolism of the two congeners is similar in monkeys and humans.

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study: Medium
Data Base: Medium
RfD: Medium

The critical study (i.e., series of studies on the same animals) rates
medium confidence. The study was well conducted in a sensitive animal species
(rhesus monkeys) that closely resemble man for many biological functions.
These studies evaluated many sensitive endpoints of PCB toxicity and the
effects observed have also been documented for occasions of human exposure.
Many sophisticated reproductive and neurological tests were performed over a 6
year time course and many clinical chemistry determinations were evaluated on
the dams during the exposure period. Very extensive analyses of feed samples
and tissue samples from dosed monkeys were performed. Although contamination
of the control laboratory primate diet with PCBs was detected, the level of
contamination was at the level of parts per billion and dosing of Aroclor 1016
was in the parts per million range. The investigators carefully documented
the levels of test material and contaminant throughout the exposure and post-
exposure period in animal tissues. Because the system of placentation,
hemothelial-chorial with bidiscoidal distribution, is similar for rhesus
monkeys and humans, it is felt that toxic events that are induced during
gestation for rhesus monkeys will be highly predictive of similar events in
humans. Historically, developmental neurobehavioral effects observed in
rhesus monkeys are predictive of similar effects in humans. Although these
studies were performed in an academic setting prior to the era of Good
Laboratory Practices- Quality Control-Quality Assurance, the study report
provides ample documentation of the experimental protocol and quality of data
collected. While the group sizes for this study are small (8 monkeys/group)
when compared to the standards for rodent studies they are within the
acceptable range for studies of large mammalian species as determined by EPA.
Medium confidence in the RfD follows.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

EPA source document - U.S. EPA. 1980. Ambient Water Quality Criteria
Document for Polychlorinated Biphenyls. Prepared by the Office of Health and
Environmental Assessment, Environmental Criteria and Assessment Office,
Cincinnati, OH for the Office of Water Regulations and Standards, Washington,
DC. EPA-440/5-80/068. NTIS PB81-117798/AS.

Other EPA Documentation - U.S. EPA. 1984. Health Effects Assessment for
Polychlorinated Biphenyls. Prepared by the Office of Health and Environmental
Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for
the Office of Emergency and R----I.A.5.

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1990).

Agency Work Group Review: ../../..

Verification Date: 6/24/92

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DRAFT

SUBJECT: RfD/RfC Work Group Meeting——February 9-11, 1993

TO: RfD/RfC Work Group

FROM: Annie M. Jarabek
            Office of Health and Environmental Assessment
            Research Triangle Park, NC

            Kenneth A. Poirier
            Office of Health and Environmental Assessment
            Cincinnati, OH

IN ATTENDANCE WERE:

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February 9, 1993

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7. **Update on the RfD for Arochlor 1016:** The RfD for Arochlor 1016 was loaded onto IRIS on January 1, 1993. Industry representatives met with Erich Bretthauer (AA, ORD) on January 6, 1993 to discuss this RfD. Mr. Bretthauer agreed that industry had some valid concerns regarding the RfD for Arochlor 1016, and recommended that this RfD be sent to the Risk Assessment Forum who will arrange for an external review of the file. .

8. **Update on IRIS Quality Assurance Team (QAT):** Annie Jarabek (OHEA/HPA) explained that the IRIS QAT was appointed by Hank Habicht. The QAT is developing a questionnaire for various IRIS users (e.g., state and local governments, environmental, and industry groups). The questionnaire is aimed at establishing how peer review of the information on IRIS might be accomplished. Work Group members will also be interviewed. A Federal Register Notice will soon be published regarding IRIS (in particular, reiterating how the IRIS Submissions Desk can be used and discussing the existence and mission of the QAT).

9. Kenneth Poirier passed out a paper by Renwick entitled "Safety factors and establishment of acceptable daily intakes" from a symposium attended by Mike Dourson (OHEA/STA) (Attachment 2).

10. Robert Beliles (OHEA/HHAG) stated that he had a problem with the atrazine RfD. He questioned why the 2-year dog study was used instead of the 2-year rat study. In addition, a developmental effect was seen at the same dose identified as the critical effect level and blood dyscrasias occurred in both rats and dogs with the LOEL in dogs being one-tenth of the rat LOEL. Rick Whiting (OPP) responded that he would bring this file back at the March meeting so that the Work Group could revisit these issues.

11. A copy of the updated IRIS Reference Dose Background Document was passed out together with a copy of the memo from the Work Group Chairs to Bill Farland requesting that the updated background document undergo appropriate review and approval for inclusion on IRIS (Attachment 3).

Aroclor 1016

John Cicmanec (OHEA/STA) passed out a memorandum regarding the RfD for Aroclor 1016 (Attachment 9). This RfD was verified on November 4, 1992 at 8E-5 mg/kg-day. In the process of loading this file onto IRIS, an error in the transposition of the NOAEL dose was noticed. The correct NOAEL is 0.007 mg/kg-day, rather than 0.008 mg/kg-day as written in the summary sheet. Correction of this mistake resulted in an RfD of 7E-5 mg/kg-day. The correct RfD was loaded onto IRIS, and the memorandum served to bring the mistake and its resolution to the attention of the entire Work Group.

BRIEFING PACKAGE

MICHAEL DOURSON

TO

JOHN SKINNER

AUGUST 2, 1992

Briefing Package 8·20-92 (AM)
Mike Dourson to John Skinner

✶ Pages given to GE at 8·20-92 (PM)
Briefing with John Skinner

Arochlor 1016 Reference Dose

Review Status of the Arochlor 1016 RfD

December 1989: Development of a arochlor 1016 IRIS file for work group discussion

February 1990: First EPA work group discussion of 1016

April 1990: Arochlor 1016 is loaded onto IRIS Service code 8 (i.e., notification of EPA discussion)

March 1991: OHEA notifies interested EPA scientists and managers and ATSDR of its intent to develop RfDs for commercial PCB mixtures

February 1992: OHEA interacts with GE scientists at Region II Hudson river site

March 1992: GE scientists send the critical study for arochlor 1016 to OHEA

March 1992: Second EPA work group discussion of 1016

May 1992: OHEA sends the results of its statistical analysis of the critical study on arochlor 1016 to GE

June 1992: Unanimous EPA work group verification of the arochlor 1016 RfD

October 1992: Anticipated IRIS load

Critical Effect: Reduced birth weights in rhesus monkeys

Studies: A series of developmental and reproductive studies in rhesus monkeys by Barsotti and colleagues

No Observed Adverse Effect Level : 0.008 mg/kg/day

Lowest Observed Adverse Effect Level: 0.03 mg/kg/day

Uncertainty and Modifying Factor: This factor is judged to be 100.

Reference Dose: 8 x 10(-5) mg/kg/day

UNCERTAINTY FACTORS
for arochlor 1016

VALUE	EXTRAPOLATION
3	AVERAGE HUMAN TO SENSITIVE HUMAN
3	ANIMAL TO HUMAN
3	SHORT TERM TO LONG TERM EXPOSURE
1	LOAEL TO NOAEL
3	MINIMUM TO COMPLETE DATA BASE

The composite factor of 100 was judged to be appropriate by the work group in June of 1992.

Confidence in the RfD

The Agency has **medium confidence** in the Reference Dose based on ...

medium confidence in the critical studies since they were well conducted and evaluated **sensitive endpoints,** and

medium confidence in the data base due to **limited** toxicity and reproductive **data**.

The available human toxicity data are consistent with the animal findings. Both together form a **plausible picture** of toxicity for arochlor 1016 in humans.

BRIEFING PACKAGE

GENERAL ELECTRIC

TO

ENVIRONMENTAL PROTECTION AGENCY

AUGUST 2, 1992

Briefing Package 8-20-92 (PM)
GE to EPA (JOHN SKINNER - ORD)

IRIS

A Primary Resource For Risk Assessors

- Basis for policy and regulatory decisions at Federal and Local levels having far-reaching impact and cost implications.

- ORD objective to "ensure that the information on IRIS is of the highest quality and represents the best risk assessment possible".

- EPA is currently considering the restructuring of IRIS to incorporate external peer review before the establishment of Cancer Potency Factors and Reference Doses.

- EPA is also proposing a new oral RfD for PCBs based on studies of Rhesus Monkeys dosed with Aroclor 1016 using work by Barsotti and Van Miller (1984) as the principal study.

The proposed establishment of a new reference dose for PCBs demonstrates the need for a new, open IRIS process that encourages and accepts broad-based scientific peer review.

Problems with Barsotti and Van Miller (1984)

<u>Sources:</u>

1)   **Barsotti, et al., 1984**

Barsotti, D.A., & Van Miller, J.P. Accumulation of a Commercial Polychlorinated Biphenyl Mixture (Aroclor 1016) in Adult Rhesus Monkeys and Their Nursing Infants. *Toxicology 30:31-44 (1984)*.

2)   **Barsotti, et al., 1976**

Barsotti, D.A., R.J. Marlar, and J.R. Allen. 1976. Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). *Fd. Cosmet. Toxicol 14:99-103*.

3)   **Barsotti Thesis**

Barsotti, D.A., Gross, Clinical and Reproductive Effects of Polychlorinated Biphenyls (PCBs) in the Rhesus Monkey. *PhD Thesis, University of Wisconsin, 1980*.

4)   **Van Miller Thesis**

Van Miller, J.P., Chemical and Pathological Observations of Chlorinated Aromatic Hydrocarbons Administered to Rats and Rhesus Monkeys. *PhD Thesis, University of Wisconsin, 1981*.

> **These studies were part of a series of experiments concerning the effects of halogenated hydrocarbons being conducted at the University of Wisconsin - Madison during the 1970's**

Study Design for Barsotti and Van Miller (1984)

**Nature of
the Study:** Sexually mature female rhesus monkeys were continuously administered a diet
containing Aroclor 1016, mated and observed through pregnancy, birth and
infant weaning. General clinical observations were recorded on adults and
offspring. Systematic determination of PCB body burden was performed.

**Study
Design:**

Species Studied:	Rhesus Monkeys
PCB used:	Aroclor 1016. (The Aroclor was reported to be devoid of chlorinated dibenzofurans.)
Sex:	Female.
Exposure Route:	Oral via diet.
Dose Groups:	Twenty four adult rhesus monkeys were assigned to one of three groups. Assignment into groups was not random.
Daily Dose:	0, 0.25, 1.0 mg/kg in the diet.
Regimen:	Daily exposure for eighty seven (plus or minus 9) weeks. During this period all animals conceived, carried to term and nursed young for 16 weeks.

Aroclor 1016 Analysis: Samples of subcutaneous adipose tissue and skin taken from females
and offspring. Milk samples collected every other week. Mesenteric
fat samples from each monkey at time of weaning.

All samples reported on a lipid basis. Neutral extraction with hexane
except for milk where diethyl ether/petroleum ether was used.

Detection by GC with a [63]Ni electron capture detector.

Problems with Barsotti and Van Miller (1984)

Selection of Controls

Control animals were not adequately matched to experimental animals. Controls were purchased in 1973 vs. 1977 for experimental animals, therefore they had 3-4 years to acclimate to laboratory conditions (reduced exercise, standard diet, reduced stress, etc.). Experimental animals had about 3-9 months acclimation, and could also have had different geographic source and age. ("Some of the animals appeared younger than others". *Barsotti thesis, p. 185).* All these uncontrolled factors could have biased the birth weight comparisons of experimentals to controls.

Dosing and Contamination Issues

1) Barsotti and Van Miller also included a dose of 0.025 mg/kg in the diet of one group of experimental animals. However, "the 0.025 Aroclor 1016 group received PBB diets for an undetermined time due to a mix-up at the pelleting site". *(Barsotti thesis, p. 186).* This dose group is not referred to in the published scientific paper.

The net effect of these two factors alone means that 50% of the original study is unsuitable for use in scientific analysis and especially in quantitative risk assessment

Problems with Barsotti and Van Miller (1984)

Dosing and Contamination Issues (cont'd)
2) PCB congeners having RRTs of 125 and 146 were present both in the milk of monkey mothers and infants' adipose tissue.

Interpretation:

- Peaks with RRT 125 and 146 represent congeners 118 and 105, resp. These congeners are not present in Aroclor 1016 but are in Aroclor 1248. Evidently some monkeys received Aroclor 1248 in diet.

- Congeners 105 and 118 are mono-ortho coplanar pentachlorobiphenyls having AHH inducing capabilities in rats.

- The presence of these congeners (or other undetected contaminants) could account for:

  - Anomalous signs of TCDD-like toxicity that developed in infants (hairline hyperpigmentation).

  - Observed birthweight and developmental deficiencies.

- Multiple toxicants were administered in these experiments.

Dosing and Contamination Issues

3) "Control adipose samples contained PCBs based on the Aroclor 1016 standard at the level of 0.69 ±0.38 ug/gm on the lipid basis" *(Barsotti thesis, p. 185)*. Three were below the limit of detection. In a subsequent publication, Barsotti stated that "pre-experimental adipose tissue samples from five of eight randomly selected animals" had PCB levels (based on Aroclor 1016) of 0.69± 0.38 ppm *(Barsotti, et al. 1984, p. 35)*.

Interpretation:

Random PCB contamination with Aroclor 1016 of control and/or experimental groups suggests contamination within the laboratory. These levels approached the levels in dosed animals.

Experimental doses are not indicative of true doses.

Dosing and Contamination Issues

4) Feed for Aroclor 1016 study was found to contain 1-50 ppb Aroclor 1248. _(Barsotti thesis, p. 185)_

5) Feed for concurrent TCDD dosing experiments contained 7 ± 4 ppb PCB. Fatty tissues (control and experimental animals) contained 20 - 2500 ppb, quantitated as either pentachlorobiphenyl or Aroclor 1248. _(Van Miller thesis, p. 97)_

Interpretation:
Contamination of feed may have occurred at feed producer, but more likely in the laboratory pelletizing operation since Aroclor 1248 was in use in concurrent experiments.

Dosing and Contamination Issues

6) "One Animal, 1641, exhibited the physical symptoms of acne and alopecia early in the experimental period. Fat analyses from the tissues of this animal revealed concentrations of PCBs that resembled the 5.0 ppm PCB animals rather than the 2.5 ppm animals". _(Barsotti thesis p. 60a)_

Interpretation:
This animal, which was meant to receive the 2.5 ppm diet, was misdosed for at least the first 12 months. _(See Barsotti thesis, Table 3-4)_

Dosing and Contamination Issues

7) Newborn offspring from nine Rhesus Monkeys fed 2.5 ppm Aroclor 1248 in diet had an average level of PCB in skin-adipose of 2.8±1.4 mg/g *(Barsotti, et al. 1976, p. 101, 102).* Newborn offspring from animals fed 1.0 ppm Aroclor 1016 had an average level of 3.37 ±0.76 ppm. *(Barsotti, et al. 1984, p. 36).*

Interpretation:

This anomaly (i.e., 2.5 x higher dose of less well metabolized PCB resulting in the accumulation a lesser amount of PCB in offspring) was probably due to erroneous dosing of either or both Aroclors in light of other study discrepancies.

Summary of Dosing and Contamination Issues

Chemicals Under Test in Univ. Wisconsin Primate Laboratories in 1970's

Aroclor 1248

Aroclor 1016

TCDD

PBB

Known Dosing/Contamination Problems

PBB in 1016 expt.

1248 in 1016 expt.

1248 in TCDD expt.

1016 in controls and/or experimentals

Overdose in 1248 expt.

Inconsistency in 1016 and 1248 offspring tissue levels

Summary of Dosing and Contamination Issues

Conclusions:

- University of Wisconsin primate laboratory procedures for chemical handling and dosing were unreliable.

- Dosage data from this laboratory are an unsuitable basis for reaching scientific conclusions or for use in quantitative risk assessment.

- Subsequent reproductive and developmental data (e.g., Levin, et al. 1988 and Schantz et al. 1989, 1991) based on animals treated in these studies cannot be reliably attributed to the chemicals administered.

- These studies are irretrievably flawed, and EPA should not rely on these studies a basis for establishing an RfD for PCBs.

CHAPTER SIX

THE UPTAKE AND ACCUMULATION OF AROCLOR 1016 IN ADULT RHESUS MONKEYS AND THEIR OFFSPRING

It has long been felt that the higher chlorinated the PCB mixtures the more potent of inducers of enzymatic activity and toxicity the PCBs were (Litterst et al.,1972; Chen and Dubois, 1973; Johnstone et al., 1974; Grote et al.,1975). The biologic activity differed between PCB mixtures and isomers. The degree of chlorination of the PCBs was related to the fate in the body. The higher the degree of chlorination the less would be excreted by the body (Matthews and Anderson, 1975; Peterson et al.,1976; Allen and Norback, 1976). Due to this property of the PCB mixtures a new product was developed that would be a good dielectric and heat transfer fluid but contained a lower percentage of the higher chlorinated PCBs. It was through that these would be less persistent in the environment. The Aroclor 1016 contains about 41% chlorine and differs from Aroclor 1242 with its 42% chlorine by weight by containing only 1/10 the level of penta- and hexachlorinated biphenyls. In feeding studies comparing the storage and distribution of Aroclors 1016 and 1242, Burse and her colleogues (1974) found that the adipose plateaus of PCBs were higher in value with Aroclor 1016. They found that the level of PCBs throughout the experimental period was higher for Aroclor 1016 in the brain, liver and urine. From studies with isomers it has been suggested that the position of the chlorines and the unsubstituted carbon atoms influence the behavior of PCBs. Unsubstituted pairs of carbons at the 3,4 positions lead to rapid elimination of the PCBs from the body (Gage and Holm, 1976).

Recently PCB isomers have been classed as two different types of enzyme inducers: phenobarbital and 3-methylcholanthrene (3-MC) types. No one isomer can be identified as both types of inducers at this time. The Aroclor mixtures are composites of both types of inducers (Goldstein,1979). The 3,3',4,4'-configuration of the biphenyl appears to be a minimum requirement for the 3-MC type inducer which is the toxic element of the PCB mixtures (Yoshimura et al.,1979). Thus the Aroclor mixtures behave in biological systems according to the types of inducers that are present and this does not necessarily depend upon the degree of chlorination but more importantly the position of the chlorines.

Little is known about the activity of Aroclor 1016 in biological systems. For this reason this reported study was undertaken to evaluate: 1) the gross, clinical and reproductive health of female rhesus monkeys fed diets containing Aroclor 1016 at the levels of 1.0, 0.25, and 0.025 ppm; 2) the storage of PCBs in the female, the pregnant female and the lactating female; 3) the accumulation of PCBs in the infants through in utero and transmammary movement of PCBs and the effects this movement of PCBs might have on the infants.

MATERIALS AND METHODS

Twenty-four adult female rhesus monkeys were procured from an importer and placed in quarantine for three months. The animals were housed in rooms whose climatic conditions mimicked that of a 20 day period in the breeding season of the animals' native India in respect to temperature, lighting and humidity. During this time the monkeys were examined daily, given food and water ad libitum and supplemented with fruit twice weekly. On the day of their arrival in the colony

and monthly thereafter during the quarantine period the animals were given intradermal tuberculin in the upper eyelid and examined 48 hours thereafter for any reaction. Body weights were taken biweekly, hemograms monthly and serum chemistry analyses (serum total lipid, cholesterol and glutamic pyruvate transaminase (SGPT)) were performed every third month. The menstrual cycles were observed and recorded as to menstrual cycle length and mensus length and intensity. Once an animal has demonstrated regularity of three cycles blood was drawn throughout an entire menstrual cycle to obtain serum for determinations of 17-β-estradiol and progesterone levels (Chapters 2 and 4). Eight females from the general colony served as controls.

Following the completion of the pretreatment evaluations the animals were divided into four groups of 8. Three of the groups were placed on the diets containing 1.0, 0.25 and 0.025 ppm Aroclor 1016 (< 5 ppb dibenzofurans, McKinney, personal communication). The remaining eight females received diets to which no additional PCB was added. The diet was prepared by adding the appropriate amount of Aroclor 1016 stock to the appropriate amount of corn oil to produce 240 ml of PCB containing oil. This was added to 50 lbs of ground Purina monkey chow (Ralston Purina, St. Louis, MO.) and mixed for 10 minutes. To this mixture approximately 2000 ml of water was added and the chow was pelleted. The bagged pellets were color coded and labeled with the concentration of the diet and the date. Samples were taken for analysis in hexane rinsed vials and labeled as to diet and date. The diets were stored frozen until used. GC analysis for PCBs on the feed was employed

(AOAC,1975).

After the animals were placed on the experimental diets, complete blood counts were performed monthly. Serum chemistry assays were done every third month. Radioimmunoassays for the levels of 17-β-estradiol and progesterone was performed on the serum obtained after 3 and 6 months on the experimental diets.

Following the completion of the above determinations, attempts were made to breed the four groups of females to control males during the appropriate time of the cycle as determined by the length of the previous mentrual cycle (Chapter 2). The females were housed with the males for 96-120 hours. This procedure continued until the female became pregnant or the breeding trial was terminated. Serum was taken from blood drawn 20 days after the mean breeding day. This was used for mouse bioassay for monkey chorionic gonadotrophin to confirm pregnancy in the case of early abortion (Wilson et al.,1972). Pregnancy was confirmed by rectal palpation of the uterus at day 35 post-mating.

When the infants were born they were weighed, abdominal skin biopsies were taken, and measurements of the head circumference as well as crown to rump lengths determined. A maternal skin and subcutaneous fat biopsy was performed at this time. Similar biopsy procedures were employed on the mother and infant after an additional three months. At one week post-partum and every other week thereafter until the infant was four months old, the infant-mother pair were separated and a milk sample was taken for PCB analysis. In addition the infants were weighed and hemograms were performed at this time. At four months of age the infants were weaned, they were given a general anesthetic and mesenteric

fat was taken at laparotomy (Appendix VI). In groups of eight, based on the parturition date, the animal were removed from the diets and subcutaneous and mesenteric fat was taken by laparotomy.

A complete necropsy was performed on all animals that died during this project. Tissues were fixed in neutral buffered formalin, dehydrated, embedded in paraffit and sectioned at 5 μ. Following deparaffination, the tissues were stained with hematoxylin and eosin and examined by light microscopy. In addition, liver, adipose and adrenal samples were taken for PCB analysis.

<div align="center">RESULTS</div>

At the time of arrival the female monkeys appeared healthy. This was substantiated by their normal hematology and serum chemistries, and the absence of positive tuberculin reactions. Some the animals appeared younger than others. This was further clarified by the irregularities in their menstrual cycles. As a result of this immaturity nine months in the controlled experimental environment were required for all the animals to establish a regular menstrual cycle.

The GC analyses of the four diets for Aroclor 1016 content were 0.700±0.130 μg/gm, 0.164±0.031 μg/gm, 0.023±0.001 μg/gm and 0.005± 0.001 μg/gm in the 1.0 ppm, 0.25 ppm, 0.025 ppm and control groups, respectively. Purina monkey chow has been found to contain 1-50 ppb Aroclor 1248. Limit of detection for feed was 0.005 μg/gm.

Control adipose samples contained PCBs based on Aroclor 1016 standard at the level of 0.69±0.38 μg/gm on the lipid basis. Three animals sampled had adipose PCB levels below the limit of confident detection for this analysis.

The amount of Aroclor 1016 accumulating in the subcutaneous tissues of the animals was 2.16±1.10 µg/gm, 1.30±0.83 µg/gm and 0.29±0.14 µg/gm after four months on the experimental diets and 5.03±3.45 µg/gm, 1.61±0.43 µg/gm and 0.35±0.16 µg/gm after seven months on the diets for the 1.0 ppm, 0.25 ppm and 0.025 ppm groups, respectively (Table 6-1). It was at this time that a contamination peak was noticed in the GC analysis for the samples in the 0.025 ppm Aroclor 1016 group. This contamination was determined to be polybrominated biphenyl (PBB). It was concluded that the 0.025 ppm Aroclor 1016 group received PBB diets for a undetermined time due to a mix up at the pelleting site. Due to this unfortunate situation the procedures at the pelleting operation were changed to avoid future reoccurrence of such a situation. For the purpose of this report the 0.025 ppm Aroclor 1016 values will be reported but this contamination by PBB will be considered.

Prior to breeding there were no changes in the food intake, general appearance, hemograms or serum chemistries. In addition the menstrual cycles of the animals were unmodified as a result of the PCB exposure (Chapter 2). The levels of the circulating serum estradiol and progesterone during the third and sixth month of the experiment were determined to be similar to those recorded prior to the administration of the diets containing PCBs.

After having consumed 8.8±1.9 mg of Aroclor 1016/kg of body weight in the 1.0 ppm group, 2.0±0.4 mg/kg in the 0.25 ppm group and 0.20 ± 0.0 mg/kg in the 0.025 group, the animals conceived following one to five breedings (Table 6-2) The reproductive capability of this group

is discussed in Chapter 2.

In addition to the maintainance of normal hemograms and serum chemistry levels, these animals had uncomplicated pregnancies. One animal from the 0.025 ppm group had a stillborn infant that resulted from malpresentation of the infant. This was not considered to be statistically significant when compared to other experimental and control groups from the colony (Chapter 2) nor was it considered PCB or PBB exposure related. There were no gross or microscopic changes in this stillborn infant that were attributable to PCB or PBB exposure. The analysis of the stillborn's tissues for Aroclor 1016 showed transplacental movement of this compound. This was also true of PBB. The adipose tissue contained 0.60 µg/gm Aroclor 1016 and the other tissues revealed the characteristic 1016 patterns but the quantitation was not possible.

At birth the infants of the 0.25 and 0.025 ppm groups were similar in size and weight to those of the control infants (Table 6-3). The infants of the 1.0 ppm females had birth weights significantly less than the other infants as determined by Student's t test (p<0.05).

Samples of the infant skin taken at birth showed levels of Aroclor 1016 on the lipid basis from 3.37±0.76 ppm in the 1.0 ppm group, 1.65± 0.84 ppm in the 0.25 ppm group and only trace amounts in the lowest dose group (Table 6-4). Maternal fat samples (lipid basis) had 2.92± 0.70 µg/gm, 1.29±0.53 µg/gm and 0.73±0.78 µg/gm Aroclor 1016 in the 1.0, 0.25 and 0.025 ppm groups, respectively. (Table 6-1). The levels of Aroclor 1016 in the adipose tissues of the mothers were similar after four and seven months on the diets and at parturition. Mesenteric fat samples taken when the adult females where removed from the diets

and their infants at weaning contained considerable PCBs. The samples obtained from the 1.0 ppm mothers contained 4.30±1.50 ppm PCB (lipid basis) while their infants' mesenteric fat contained 27.54±7.19 ppm PCB (lipid basis). The 0.25 ppm mothers showed levels of 1.50±0.53 ppm (lipid basis) their infants showed levels of 10.39±3.69 ppm PCB (lipid basis). The 0.025 ppm group was considerably lower (Table 6-1 and 6-4). Milk fat levels of PCB obtained biweekly throughout the four months of nursing averaged 3.44±0.31 ppm, 1.47±0.37 ppm and trace levels for the 1.0, 0.25 and 0.025 ppm groups, respectively (Table 6-5).

When the adults were removed from the experimental diets no gross hematological or clinical chemistry alterations were observed (Tables 6-6 and 6-7) The animals maintained normal food consumption and body weights throughout the experimental period (Tables 6-2 and 6-8).

During the four months of nursing all the infants showed consistent weight gain, however, the infants from the 1.0 ppm group did not attain body weights equal to those of the other groups (Table 6-3). In addition to the differences in weights four of the eight infants from the 1.0 ppm group developed marked hyperpigmentation of the skin about the hairline of the face and down the middle of the scalp. Similar changes in skin color were observed in the other two groups to varying degrees. Hematological determinations conducted on the infants during the course of nursing were similar to those recorded in the control infants (Table 6-9). The total intake of Aroclor 1016 by the adult females at the time the infants were weaned was 19.1±4.4 mg/kg for the 1.0 ppm group, 4.6±0.6 mg/kg for the 0.25 ppm group and 0.5±0.1 mg/kg for the 0.025 ppm group (Table 6-2).

Analysis of Aroclor 1016 levels in the tissues of the adults and
infants as well as the mother's milk indicated transplacental and
mammary movement of the PCBs. The accumulation of Aroclor 1016 in the
adults did not include the total spectrum of peaks observed in the
Aroclor 1016 standard (Figure 6-1). There was a preferential accumula-
tion of three peaks with relative retention times (RRT) of 37, 47 and
70 (relative to DDE) representing approximately 90% of the Aroclor 1016
present (Figure 6-2). These three peaks represent approximately 45%
of the standard for Aroclor 1016.

The infants at birth showed an accumulation of all the Aroclor 1016
peaks in their skin. The peaks with RRT's of 37, 47 and 70 comprised
the majority of the PCB's representing approximately 80% of the Aroclor
1016 present. By the end of the nursing period (four months of age)
mesenteric fat biopsies from the infants showed an accumulation of
Aroclor 1016 peaks similar to that in the adults (Figure 6-3). However,
the contribution of the peaks with RRT's of 37 and 70 was greater in
the infants than the adults. These two peaks represented approximately
80% of the total Aroclor 1016 present in the infants as compared to
approximately 60% in the adults. The peak with RRT of 47 comprised
only 5-10% of the Aroclor 1016 in the infants but comprised approxima-
tely 25% in the adult. Four months after weaning the pattern of Aroclor
1016 peaks in the mesenteric fat of the infants was similar to that
observed at the time of weaning although the total levels had decreased
(Table 6-4).

The analyses of milk samples indicated that almost all of the Aroclor
1016 peaks were excreted via this lactational process (Figure 6-5).

The relative quantities of the individual peaks were different from the standard with peaks with RRT's of 37 and 70 representing a greater percentage of the total.

In almost all of the GLC analyses, two peaks with RRT's of 125 and 146 were observed (Figures 6-2,6-3,6-4). These peaks cochromatographed with two of the major peaks observed in commercial mixtures of higher chlorine content. These peaks were also seen in samples from control animals (Figure 6-6). In milk samples, these peaks were frequently masked by a large peak presumably due to lipid which was not removed during cleanup procedures (Figure 6-5).

DISCUSSION

In the past it has been proposed that the lower chlorinated PCB mixtures such as Aroclor 1016 would be more rapidly metabolized and thus be less toxic than the higher chlorinated mixtures. There are indications from the presently reported study that the response of monkeys chronically exposed to low levels of Aroclor 1016 are similar to those observed when low levels of Aroclor 1248 was employed (Chapter 5). Thus it would appear that there are circumstances were the toxic manifestations produced by the lower chlorinated PCB mixtures are similar to those caused by the mixtures comprised of more highly chlorinated species.

The preferential accumulation of the 3 peaks presumably indicates the ability of the nonhuman primate to metabolize and excrete some of the compounds in the Aroclor 1016 mixture more readily than others. The accumulation of the peak with RRT is surprising based on the iden-

tification of this peak as a trichlorobiphenyl by Sawyer (1978 a,b).
All previous studies have indicated that trichlorobiphenyls are read-
ily metabolized and excreted by birds and mammals (Matthews et al.,
1978). A recent report of the Yusho women exposed to the contamina-
ted rice oil indicated that the milk contained PCB residues with tri-
and tetrachlorinated species. The specific structure of the residual
components was 4,4'-substitution pattern (Yakushiji et al.,1979).

The identification of almost all of the Aroclor 1016 peaks in the
skin of the neonates presumably indicates the inability of the fetus
to metabolize the compounds which are easily metabolized in adults
and older infants. This conclusion is further substantiated by a
loss of these peaks in the infants even while nursing. The decrease
in Aroclor 1016 levels in the infants after weaning is not readily
explained. Based on the accumulation of certain peaks in the adults
it would seem unlikely that extensive metabolism of these peaks would
occur in the infants. This possibility cannot be ruled out. However,
another explanation may be that the decrease in PCB concentration may
be due to an increase of size with a subsequent dilution of the PCBs
without excretion. A third possible explanation for these findings
is that during the rapid period of growth, the dynamic shift in major
anabolic pathways may alter the storage, distribution and elimination
of the PCBs. The understanding of these results is further complica-
ted by the differences observed in relative quantities of certain
peaks between the infants and adults. The importance of the accum-
ulation or metabolism of certain compounds on the toxic effects that

are induced in these animals cannot be evaluated on the basis of the information that is available.

Of particular interest is the finding of the multiple peaks of Aroclor 1016 in the mothers' milk. This observation may indicate that ingested PCBs are readily transferred to milk fat during periods of heavy lactation before significant metabolism can occur. The possibility that in utero and mammary exposure of the infants to the compounds that are readily metabolized by the adult may have causative relation on the intoxication that developes in the infant. Studies to date have not elucidated the compounds responsible for toxicity in the mixtures that are employed. In the light of Goldstein's (1979) and Yoshimura's (1979) observations as to the nature of the isomers present in the mixtures, identification of the isomers with the RRT's mentioned previously and their classification as to the type of inducers would be important. This information is presently being sought.

The finding of higher chlorinated PCBs in control and experimental samples is not surprising since PCBs have been shown to be ubiquitous in the environment. They have been found in commercial monkey chow also (Coleman and Tardiff, 1979). We have shown that the concentration of PCBs in control monkey show is in the range of 1-50 ppb on the basis of an Aroclor 1248 standard. Incidental findings of PCB levels up to 1.0 ppm in fat samples of monkeys from our colony are not unusual. These levels however did not affect infants from control animals or the reproductive capabilities of the adults (Chapter 2). These may be nontoxic congeners which are stored in the adipose

tissue of mammals.

In contrast to the severe signs of intoxication observed in the infants that received larger doses of PCBs (Chapter 5) the adult females which were employed in the present study did not show any overt signs of PCB intoxication. In addition their general body health, food consumption, body weights, hematology, serum chemistry and reproductive processes were unaltered when comped with the control monkeys from our colony (Chapter 2). The unfortunate PBB contamination of the 0.025 ppm PCB group did not appear to alter any of the toxic parameters, However, this cannot be concluded until the behavioral evaluation of the infants is completed.

Even though the adult animals were normal in all of the parameters evaluated such was not the case with their infants. In addition to the 1.0 ppm PCB infants being smaller and showing difficulty in weaning (one infant died from the stress of maternal separation) Six of the 8 infants of the 1.0 ppm group, 1 of the 8 infants of the 0.25 ppm group and 2 of the 7 infants from the 0.025 ppm group developed hyperpigmentation. These changes were similar to those described in the infants that were exposed to Aroclor 1248 (Chapter 5).

It appears that levels of Aroclor 1016 which range from 1.0 to 0.25 ppm (the 0.025 ppm group would be excluded from this discussion for the above stated reasons) will not produce any overt signs of intoxication even when the period of exposure exceeds 18 months in the adult female monkeys. However when infants are born to these seemingly normal adult monkeys,they will exhibit manifestations of

intoxication. There is sufficient PCB exposure via transplacental
and secretion during lactation by the mothers to produce signs of PCB
intoxication in the infants.. The highest level of PCB consumption
occir during the four months of nursing with a concomitant increase
in body burden of PCBs.

The effects of PCB accumulation in ths tissues of the infants are
subtle and mild manifestations.of intoxication could be overlooked
easily.if they ocurred in a colony were these particular parameters
were not being evaluated crtically.

In the light of the ubiquitous distribution of PCBs in our en-
vironment at the present time, their presence in the food chain, of
human consumption, their detectability in ppm concentrations in the
human adipose tissues and the manifestation of experimental intoxica-
tion of primates at dose levels of ppb, it would seem that judicious
concern needs to be afforded to this important environmental contam-
inant.This study suggests such evaluation will probably require more
exacting procedures than are presently available to detect minimal
signs and symptoms of intoxication in animals and man.

FIGURE 6-1 Gas chromatographic tracing of 1 ng Aroclor 1016 FDA
standard 1151 (77029). Attenuation = 64, Temperature =
200° C. The numbers assigned to the peaks designate the
relative retention times with reference to p,p'-DDE as
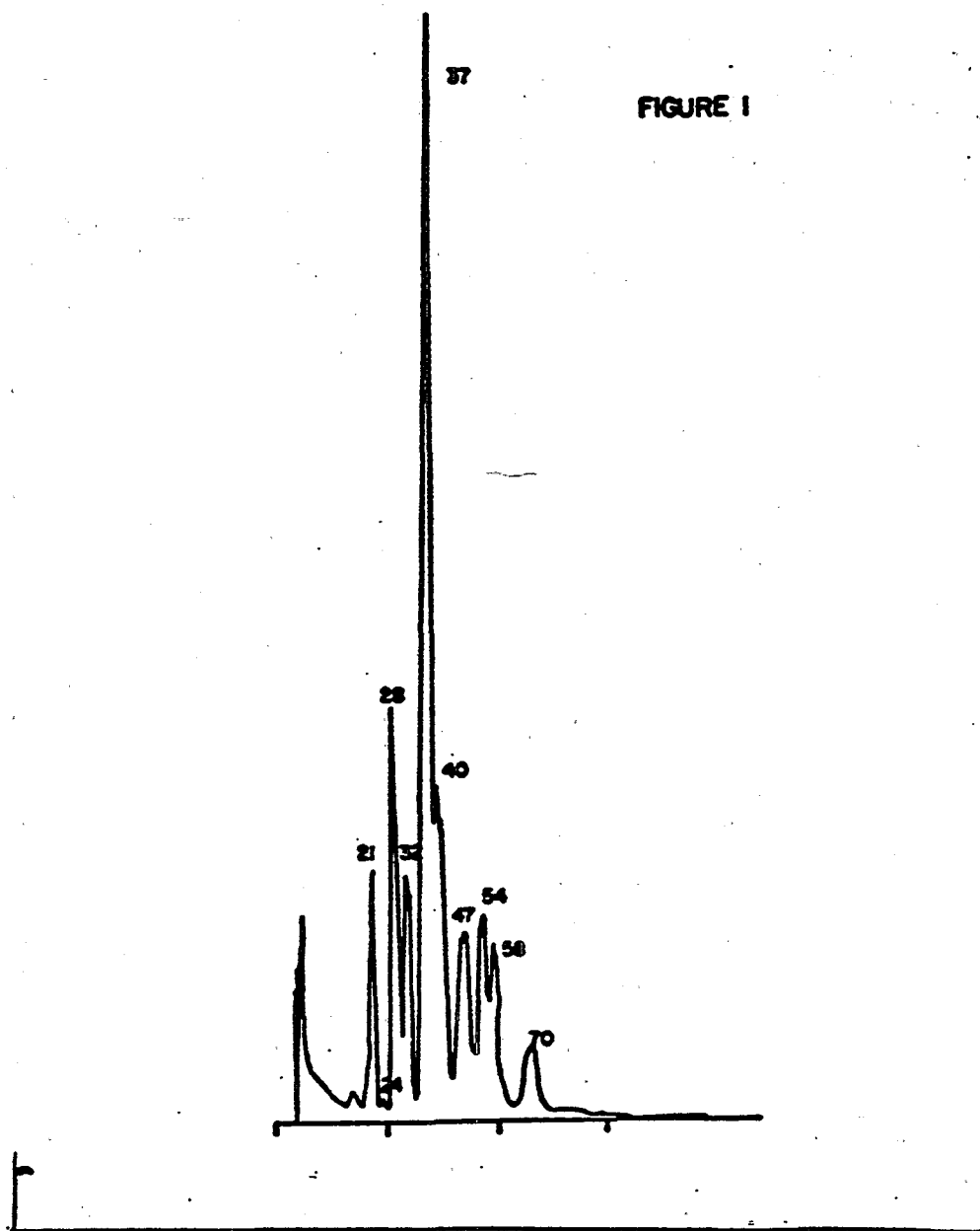100.

FIGURE 1

FIGURE 6-2   Gas chromatographic tracing of a subcutaneous fat sample
from monkey #79 after receiving Aroclor 1016 in the diet
at 1.0 ppm for 18 weeks. Attenuation = 8; Temperature =
200°C. The numbers assigned to the peaks designate the
relative retention times with reference to p,p'-DDE as
100. The level of Aroclor 1016 on the whole tissue basis
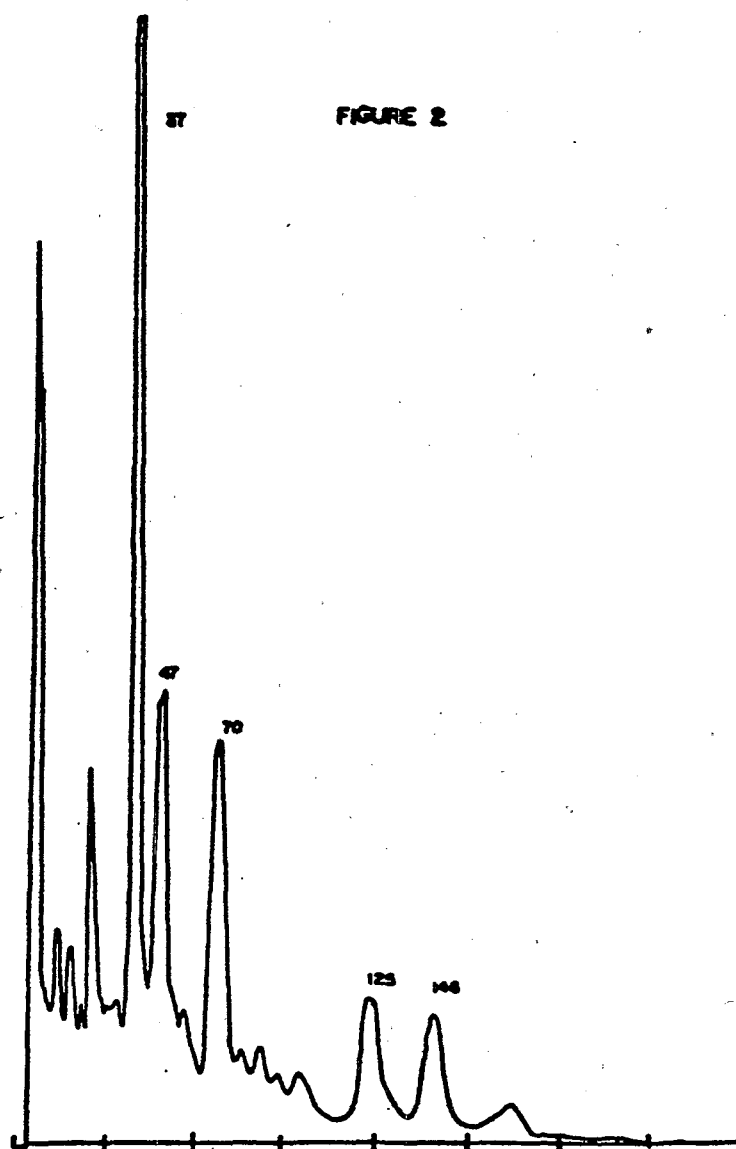was 2.27 µg/gm and 3.25 µg/gm on a lipid basis.

FIGURE 2

FIGURE 6-3   Gas chromatographic tracing of a mesenteric fat sample
taken from monkey #79's infant AG-81 (1.0 ppm Aroclor
1016) at the time of weaning from the mother. Attenua-
tion = 32; Temperature = 200° C. The numbers assigned
to the peaks designate the relative retention times with
reference to p,p'-DDE as 100. The level of Aroclor 1016
on a whole tissue basis was 10.43 µg/gm and 31.31 µg/gm
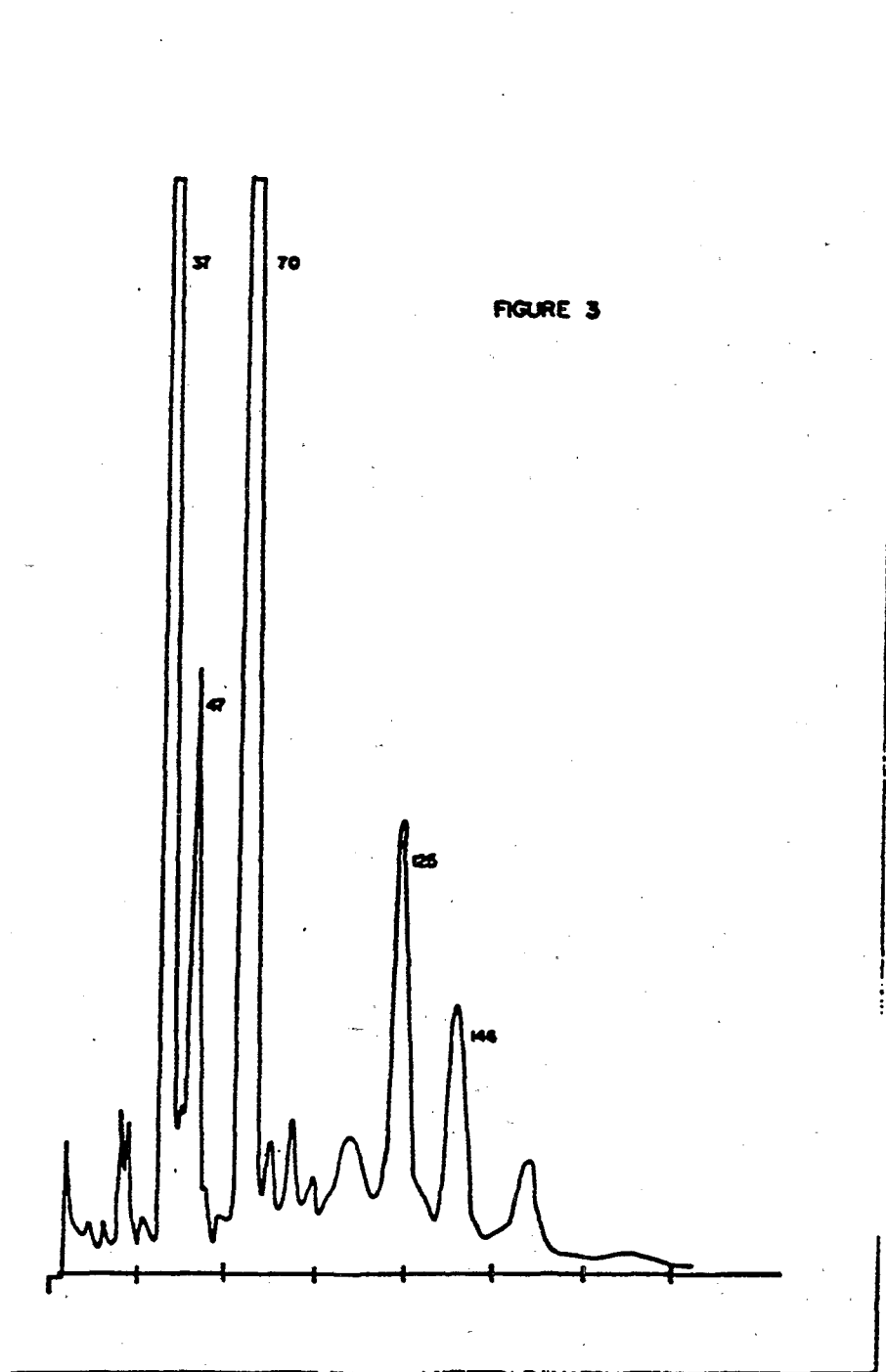on a lipid basis.

FIGURE 3

FIGURE 6-4   Gas chromatographic tracing of a mesenteric fat sample
taken from monkey #79's infant AG-81 (1.0 ppm Aroclor
1016) four months after weaning from the mother. Atten-
uation = 8; Temperature = 200° C. The numbers assigned
to the peaks designate the relative retention times with
reference to p,p'-DDE as 100. The level of Aroclor 1016
on a whole tissue basis was 1.96 µg/gm and 2.96 µg/gm on
a lipid basis. Note that peak 47 has been partially
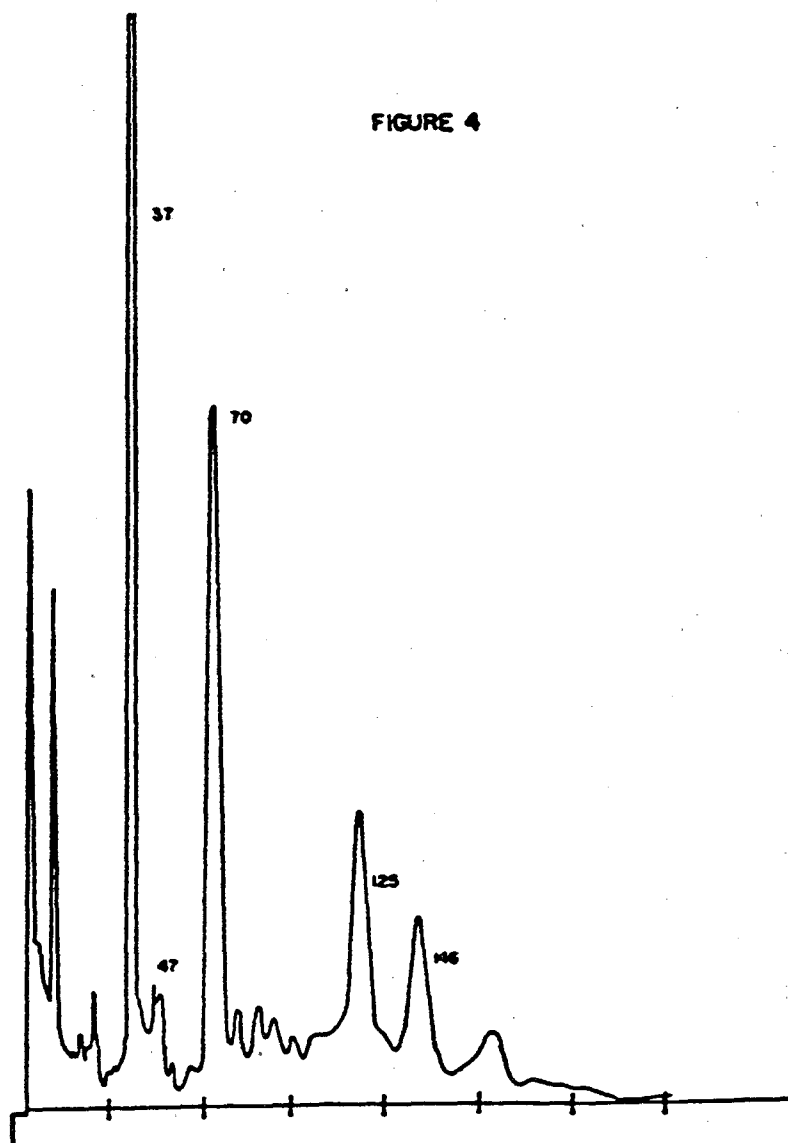resolved into two parts when compared to Figure 6-3.

FIGURE 4

FIGURE 6-5 Gas chromatographic tracing of a milk sample taken from monkey #79 (1.0 ppm Aroclor 1016) during the nursing period. Attenuation = 8; Temperature = 200^0 C. The numbers assigned to the peaks designate the relative retention times with reference to p,p'-DDE as 100. The level of Aroclor 1016 on a whole milk basis was 0.11 µg/gm and 2.99 µg/gm on a lipid basis.
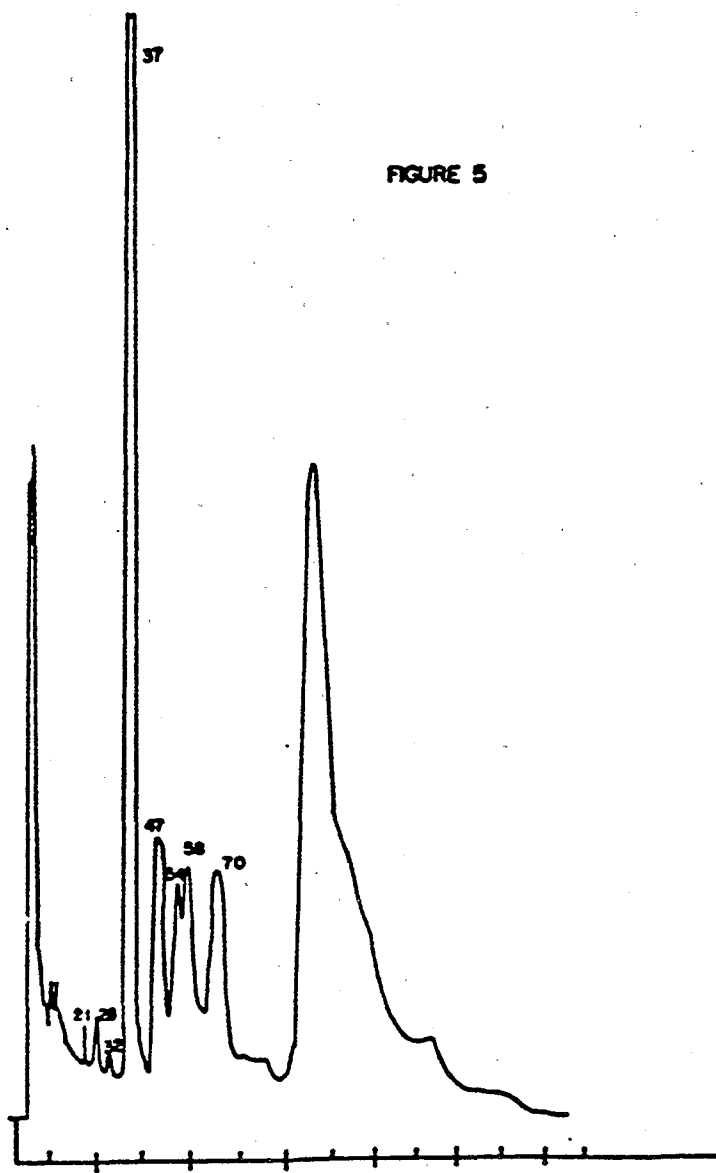
FIGURE 5

FIGURE 6-6   Gas chromatographic tracing of a subcutaneous fat sample
taken from monkey #33 (control) at parturition of her
infant. Attenuation = 16; Temperature = 210° C. The
numbers assigned to the peaks designate the relative
retention times with reference to p,p'-DDE as 100. The
level of Aroclor 1016 on a whole tissue basis was 0.36
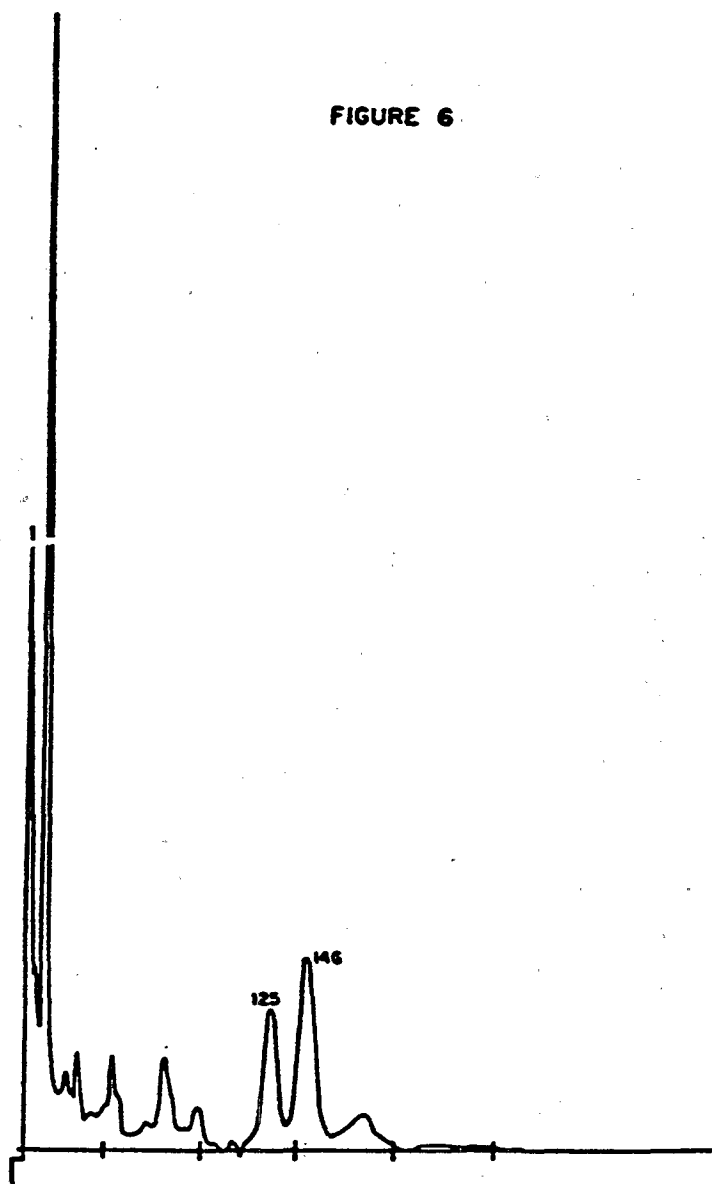µg/gm and 0.40 µg/gm on a lipid basis.

FIGURE 6

TABLE 6-1 Levels of PCBs (Fat Basis) in the Adipose of Female Monkeys Consuming a Diet Containing Aroclor 1016

Levels in diets (μg/gm)	After 4 mo. on diet (μg/gm)	After 7 mo. on diet (μg/gm)	At parturition (μg/gm)	At Weaning (μg/gm)
1.0	2.16±1.10 (n=5)	5.03±3.54 (n=6)	2.92±0.70 (n=8)	4.30±1.50 (n=7)
0.25	1.30±0.83 (n=8)	1.61±0.43 (n=6)	1.29±0.53 (n=8)	1.50±0.53 (n=5)
0.025	0.29±0.14 (n=5)	0.35±0.16 (n=6)	*0.73±0.78 (n=7)	*0.61±0.29 (n=5)
Control	----	----	*0.37±0.16 (n=6)	----

* 2 samples were below the limit of detection (0.02 μg/gm)
Values = Mean±1 standard deviation

TABLE 6-2 Intake of Aroclor 1016 by Adult Female Rhesus Monkeys

Level in diet (µg/gm)	Intake after 7 months (mg/kg)	Intake at Conception (mg/kg)	Intake at Parturition (mg/kg)	Intake at Weaning (mg/kg)
1.0	6.1±0.9	8.9±1.9	10.8±2.1	18.1±3.1
0.25	1.7±0.3	2.0±0.4	2.6±0.3	4.5±0.6
0.025	0.2±0.0	0.2±0.0	0.3±0.0	0.5±0.1

Values = Means±1 standard deviation

TABLE 6-3 Weights and Measurements of Infants whose Mothers were
Exposed to Aroclor 1016 prior to and during Pregnancy
and Lactation

Level in diet (μg/gm)	Weight at Birth (gm)	Weight at 17 wks of age (gm)	Head Circumference (cm)	Crown to Rump Length (cm)
1.0	422±27	864±97	19.1±0.51	15.7±0.9
0.25	491±23	939±72	19.3±0.45	16.5±0.8
0.025	489±54	939±59	19.2±0.47	16.9±1.0
Control	512±64	896±90	19.5±0.50	16.8±1.3

Values = Means± 1 standard deviation

TABLE 6-4   PCB Levels (Fat Basis) in the Tissues of Infants whose
            Mothers Consumed Aroclor 1016

Level in Diet (µg/gm)	At Birth Skin (µg/gm)	4 mo. of Age Mesenteric Fat (µg/gm)	8 mo. of Age Mesenteric Fat (µg/gm)
1.0	3.37±0.76 (n=8)	27.51±7.19 (n=8)	3.75±1.00 (n=5)
0.25	1.65±0.84 (n=7)	10.39±3.69 (n=8)	1.96±0.54 (n=5)
0.025	*0.23 (n=3)	2.74±0.47 (n=7)	**0.61±0.30 (n=6)
Control	***1.0.;2.07	----	0.46±0.16 (n=6)

*2/3 samples were below the limit of detection for this analysis
        (0.02 µg/gm)
** 1/6 samples was below the limit of detection for this analysis
*** 4/6 samples were below the limit of detection for this analysis

Values = Means 1 standard deviation

TABLE 6-5 PCBs in Breast Milk (Lipid Basis) from Female Rhesus Monkeys that have Consumed Diets Containing Aroclor 1016

Level in diet (μg/gm)	During 1st mo. lactation (μg/gm)	During 2nd mo. lactation (μg/gm)	During 3rd mo. lactation (μg/gm)	During 4th mo. lactation (μg/gm)
1.0	3.00±0.60 (n=8)	3.70±0.94 (n=7)	3.46±1.19 (n=8)	3.60±0.88 (n=8)
0.25	1.08±0.35 (n=8)	1.23±0.17 (n=8)	1.74±0.53 (n=8)	1.83±0.75 (n=8)
0.025	*0.69 (n=6)	N.D. (n=6)	N.D. (n=6)	**0.48 (n=7)
Control	***0.49,0.45, 1.07 (n=8)	0.94 (n=8)	****0.62,1.02 (n=8)	****0.40,1.06 (n=8)

*5/6 samples were below the level of detection
** 6/7 samples were below the level of detection
*** 5/8 samples were below the level of detection
**** 6/8 samples were below the level of detection

N.D.= not detectable, below the level of detection (0.02 μg/gm)
Values = Means±1 standard deviation

TABLE 6-6 Hemograms of Female Monkeys Exposed to Aroclor 1016 for
Approximately Twenty Months

Level in diet (μg/gm)	Hematocrit %		Hemoglobin (g/dl)		White Blood Cell (x 10^3)	
	Initial	20 months	Initial	20 months	Initial	20 months
1.0	38±5	41±3	12.6±1.7	12.8±0.9	7.6±2.5	8.2±1.5
0.25	41±2	40±2	13.5±0.6	13.4±1.3	11.2±4.2	8.9±2.4
0.025	36±7	40±2	12.1±2.2	12.5±1.2	11.2±2.5	8.3±2.6

Values = Mean±1 standard deviation

TABLE 6-7   Serum Chemistry Determinations of Female Rhesus Monkeys
             Exposed to Aroclor 1016 for Approximately Twenty Months

Level in diet (µg/gm)	Total Lipid (mg/dl)		SGPT (units)		Cholesterol (mg/dl)	
	Initial	Final	Initial	Final	Initial	Final
1.0	433±70	415±133	9.4±9.1	13.3±6.8	150±32	162±11
0.25	460±103	390±94	17.6±7.5	11.4±3.2	155±33	156±41
0.025	428±79	446±123	15.3±8.4	14.3±5.5	136±25	140±21

Values = Mean±1 standard deviation

TABLE 6-8 Body Weights of Adult Female Rhesus Monkeys that Consumed a Diet Containing Aroclor 1016 for Approximately Twenty Months

Level in diet (μg/gm)	Initial Body Weights (kg)	Body Weight after Weaning (kg)
1.0	5.04±1.07	5.18±0.95
0.25	4.88±0.60	5.47±0.80
0.025	4.85±0.76	5.30±0.75

Values = Mean±1 standard deviation

Table 6-9 Hemograms of Infant Monkeys whose Mothers were Exposed to Aroclor 1016 prior to and During Pregnancy and Subsequent Lactation

Level in diet (μg/gm)	Hgb (g/dl)	1 week Hct (%)	WBC (x 10³)	Hgb (g/dl)	5 weeks Hct (%)	WBC (x 10³)	Hgb (g/dl)	12 weeks Hct (%)	WBC (x 10³)
1.0	15.9±0.8	46.5±3.3	6.0±1.6	13.0±0.2	39.4±1.3	7.5±2.2	12.3±0.5	37.7±2.1	10.6±1.7
0.25	15.6±1.0	46.2±3.2	6.3±1.9	13.2±1.8	39.2±2.0	6.3±1.9	12.4±0.9	37.7±3.3	9.5±3.7
0.025	16.2±1.9	46.9±5.5	6.9±1.0	12.8±0.8	38.5±2.8	9.1±3.5	12.2±1.1	38.3±0.8	11.9±2.7
Control	15.9±0.9	47.8±3.6	6.2±2.0	13.5±0.2	39.1±2.9	6.9±1.0	12.4±0.1	38.3±1.2	8.2±1.0

Values = Mean±1 standard deviation

Date   :   April 6, 1991

To   :   Dr. John L. Cicmanec,
                ECAO

From   :   Amy Feng, Sr. Statistician
                Computer Sciences Corporation

Subject :   Statistical Analysis of PCB (1016) Dosed Monkey Birth Weight Data.

Results :   A statistically significant difference in birth weights was found
                between control and high dose (1000 ppb) groups ($p=0.003$). A
                statistically significant difference was also found between sex birth
                weights regardless of dose ($p=0.0496$). No statistically significant
                difference was found in birth weights among gestation length
                ($p=0.675$).

The PCB (1016) dosed monkeys' birth weight data were analyzed using a two-factor (sex, dose) analysis of variance (ANOVA) followed by Tukey's multiple comparison procedure to test for a dose-related effect. Initially, gestation length and the monkey sire ID were included in the ANOVA model. Gestation length was not reported for the control group. Therefore, a three way (SEX, DOSE, SIRE ID) ANOVA with gestation days as covariate was performed for only the dosed groups. No statistically significant effect of gestation length was detected. Then, the ANOVA model including DOSE, SEX, DOSE*SEX, and SIRE ID was tested. No statistically significant difference was found for SIRE ID and the interaction DOSE*SEX. The final model, then, included only DOSE and SEX as explanatory variables for the dependent variable, birth weight.

The necessary assumption of ANOVA procedure was tested and met; Bartlett's statistics for the homogeneity of variance and Shapiro-Wilk statistics for the normality of data.

Tables of summary statistics and test results are included. Please let me know if you need further information.

cc. RH
    LK

PCB (1016) Dosed Monkeys' Birth Weight Data

Table 1.   Summary Statistics

Dose (ppb) SEX	Mean Birth Weight	N	STD	MAX	MIN
0	521.11	9	64.60	595	400
25	485.00	8	60.42	560	380
250	491.25	8	24.16	525	450
1000	422.50	8	28.91	480	385
F	463.33	18	48.51	550	380
M	502.67	15	64.94	595	385

Table 2.   Analysis of Variance

Factor	ANOVA[#] p-value	Tukey's Multiple[*] Comparison Procedure	
SEX	0.0496[1]	F	M
DOSE	0.0025[1]	1000 25 250 0	
GESTATION LENGTH	0.675[2]		
SIRE ID	0.156[3]		
DOSE*SEX	0.291[3]		

[#] Two-tailed p-value from analysis of variance (ANOVA).

[*] Dose (SEX) groups connected by solid line are not statistically different ($\alpha = 0.05$). Dose (SEX) groups are arranged in order of increasing mean birth weight.

[1] Two-way (SEX, DOSE) ANOVA, no interaction term included.

[2] Three-way (SEX, DOSE, and SIRE ID) ANOVA, gestation length as covariate, no interaction term included, excluding the control group.

[3] Three-way (SEX, DOSE, and SIRE ID) ANOVA, all interaction terms included.

PCB (1016) Subjects in 1978 Experiment

| Subject No.	Sex	DOB	Birth Wt. (gm)	Gest. (days)	Mother	Father	
Controls							
AG63	F	4/22/78	510	#	PP12	PP06	
AG65	M	4/23/78	565	#	PP13	PP02	
AG66	F	4/26/78	550	#	PP14	PP01	
AG68	F	5/3/78	400	#	PP18	1630	
AG70	M	5/4/78	460	#	PP33	PP05	
AG71	M	5/8/78	595	#	PP37	PP02	
AG74	M	5/27/78	495	#	PP48	1630	
AG88	M	8/13/78	570	#	PP74	PP06	
AG96	F	9/28/78	445	#	PP66	PP02	
1ppm							
AG81	F	7/7/78	430	169	PP79	1632 **	
AG85	F	7/28/78	410	164	PP88	PP01	
AG90	M	8/30/78	480	164	PP89	PP05	
AG92	M	9/2/78	385	152	PP70	PP02	died 1/3/79
AG93	F	9/9/78	440	185	PP87	PP05	
AH02	M	10/6/78	405	163	PP76	PP01 @	
AH05	F	10/18/78	425	169	PP81	PP04	
AH14	F	12/19/78	405	151	PP54	PP04	
250ppb							
AG77	F	5/27/78	480	169	PP83	PP03	
AG79	F	7/5/78	495	163	PP82	PP01	
AG87	F	8/9/78	470	162	PP56	PP04	
AG94	F	9/14/78	515	171	PP75	PP06	
AG97	F	9/27/78	495	166	PP62	PP03	
AH03	M	10/12/78	450	164	PP67	PP03 @	
AH04	F	10/14/78	525	161	PP72	1632 **	
AH13	M	12/5/78	500	185	PP78	PP05	
25ppb							
I-1	M	6/25/78	540	155	PP77	1632 **	still-birth
AG78	M	7/5/78	535	166	PP69	PP06	
AG80	F	7/6/78	380	164	PP64	1632 **	
AG83	F	7/22/78	475	173	PP80	PP06	
AG84	M	7/25/78	470	167	PP73	PP06	
AG89	F	8/29/78	490	167	PP56	PP06	
AG99	M	9/26/78	560	161	PP85	PP05	
AH09	M	11/14/78	430	162	PP68	PP06	

* Father 1630 received 5ppm PCB (1248) in diet (12/1/73 - 1/13/75), total of 450 mg PCB
** Father 1632 received 5ppm PCB (1248) in diet (12/1/73 - 4/28/75), total not calculated
\# Gestation data for individual control Ss have not been found. However, a Biotron record
   (where they were born) gives a mean of 164.7 days with a standard deviation of ±3.5 days.
@ AH02 & AH03, listed as females at Biotron, revealed as males at the Primate Lab.

PCB (1016) Dosed Monkey Birth Weight Data
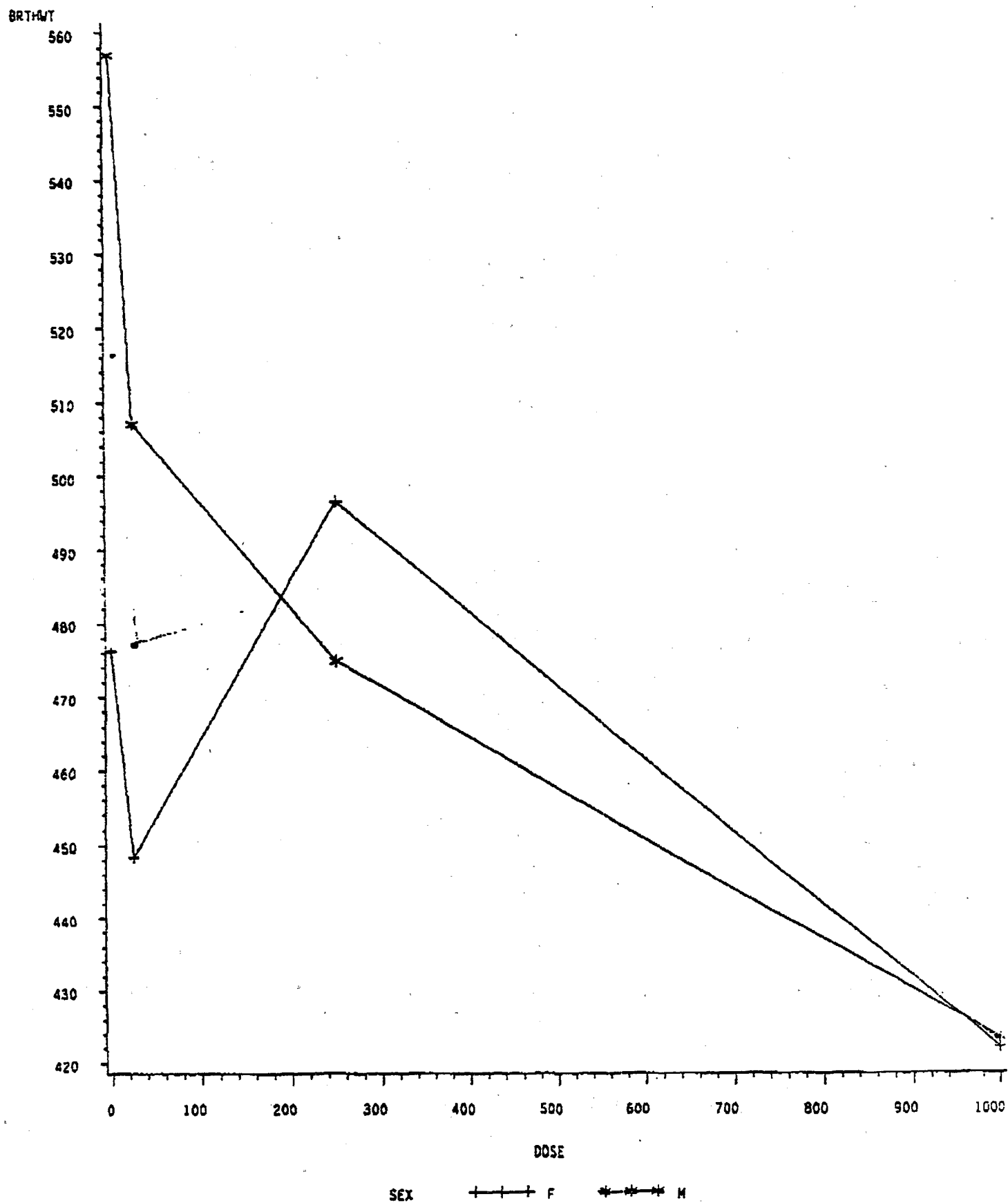
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

DATE: May 20, 1992

SUBJECT: RfD for Araclor 1016

FROM: R.C. MacPhail, Chief
           Neurobehavioral Toxicology Branch/NTD   (MD-74D)

TO: J.L. Cicmanec, Member
           RfD Workgroup Committee

        I have carefully read the materials you supplied on the
proposed RfD for Araclor 1016. While in general I agree with the
derivation of the RfD and appraisal of the supporting literature,
I do have some specific comments to make regarding the package.

        First, there is an inconsistency in the body-weight data of
the infants born to mothers exposed to 1.0 ppm Araclor 1016. The
Barsotti and van Miller (1984) paper states that the mean weight
was 422 ± 29 g. I could not find in that article whether the
variability measure was an SE or SD. In Schantz et al. (1989),
however, the weight is given as 442 ± 29 g. While here, too, the
variability estimate is uncertain, the first experiment in
Schantz et al. (1989) on Araclor 1248 presents birth weights as
mean ± SD. I believe therefore the variability estimates are
SDs, but cannot be certain. This does not resolve the problem,
however, of discrepancies in the birth weights of the 1.0 ppm-
exposed monkeys in the two publications.

        Please also note that the dose rates given on p. 2 of the
RfD write-up are slightly different from those based on the data
given in Schantz et al. (1989; see p. 247). When rounded, the
dose rate for the 0.25 ppm monkeys would be 0.008 mg/kg/d (not
0.007 mg/kg/d), and for the 1.0 ppm monkeys it would be 0.030
mg/kg/d (not 0.028 mg/kg/d).

        With regard to the behavioral data, please note that the
group sizes were 6, 6 and 6 monkeys exposed to no added Araclor
1016, 0.25 or 1.0 ppm Araclor 1016, respectively. The write-up
on p. 2 indicates 7 monkeys were included in the 0.25-ppm group.
Also, on p. 2 (2nd para. line 15) it should be noted that there
was no effect of Araclor 1016 on the position or the color
discrimination-and-reversal task.

2

As regards the Levin et al. (1988) results, neither the low dose nor the high-dose Araclor 1016-exposed monkeys differed from controls in the delayed spatial alternation task, but the two groups differed from each other. Accuracy was significantly higher in the low-dose monkeys, suggesting a biphasic effect of Araclor 1016 on performance accuracy. It should be noted that biphasic effects are not uncommon in neurobehavioral toxicology; indeed, the authors reported obtaining a similar biphasic effect with lead. It is, however, generally considered inappropriate to conclude that such an effect exists in the absence of statistically reliable treatment differences from controls. I therefore agree with your appraisal of the results of this study. As a minor point, however, it should be noted on p. 3 that the authors believed the greater accuracy in the low-dose monkey was also due to an attentional deficit (i.e., they attended to fewer irrelevant stimuli and therefore performed the task more accurately than controls).

Finally, if the high dose from Schantz et al. (1989) is accepted as the LOAEL, wouldn't the low dose be, by definition, the NOAEL?

I hope these comments are useful. I must say that I found these difficult articles to "digest," as key pieces of data were left out of many of the papers and could only be gotten by cross-referencing the papers. It is not clear to me how many experimental animals were studied in all the tests that have been published! Please do not hesitate to contact me if I may be of further service.

cc: L. Hall
    H. Tilson

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
CINCINNATI. OHIO 45268

May 12, 1994

Ms. Clare W. Stine
Mail Stop 8101
U.S. EPA
401 M St., SW
Washington, D.C. 20460

Dear Ms. Stine:

In preparation for the Aroclor 1016 Workshop which will be held later this month I would like to provide information regarding normal body weight ranges for adult female rhesus monkeys (Macaca mulatta) as well as birth weight data for infant female and male rhesus monkeys.

The sources I have been able to draw from are data collected at Litton Bionetics Kensington, MD., a commercial animal research laboratory where I was employed from 1972 to 1982, and the Wisconsin Regional Primate Research Center which includes some data for the Biotron facility where the Barsotti studies were conducted.

Laboratory	Adult Females	Birth Weights Females	Birth Weights Males
	(kg.)	(gms.)	(gms.)
Litton	5.1 +/-1.2	474 +/-54	504 +/-23
Wisconsin	4.5-7.5 (range) 5.3 (mean)	468 +/-45	494 +/-30

The data sources for the Litton Bionetics material are "Management of a Laboratory Breeding Colony Macaca mulatta", D.A. Valerio, R. L. Miller, J. R. M. Innes, K. D. Courtney, A. J. Pallotta and R. M. Guttmacher, Academic Press, New York, 1969. and some study reports that I had available. These data are taken from approximately 400 breeding females and 3000 laboratory-born infants. The information for the Bionetics colony was obtained for a period prior to and concurrent with the Barsotti study. The source for the Wisconsin data are personal conversations with Dr. Dan Houser, the veterinarian for the Wisconsin Primate Center. At the time of the Barsotti study the Biotron facility was not under Dr. Houser's care but for a later period it was under his care, therefore this information is for a later period than the Barsotti study. The data is taken from representative groups of monkeys but not all breeders and newborns for the Wisconsin Primate Center and the Biotron facility. I realize that this information does not
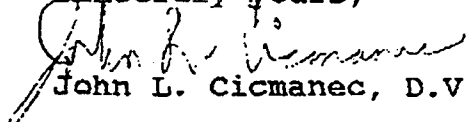
pertain directly to Biotron colony averages at the time of the Barsotti study but it is the best that I can obtain and it should serve as some basis for comparison with the experimental test groups.

Sincerely yours,

John L. Cicmanec, D.V.M.

Publications Made Available to Technical Reviewers

D. Barsotti and J. van Miller. 1984. Accumulation of a commercial polychlorinated biphenyl mixture (Aroclor 1016) in adult rhesus monkeys and their nursing infants. Toxicology, 30: 31-44.

E. Levin, S. Schantz, and R. Bowman. 1988 Delayed spatial alteration deficits resulting from perinatal PCB exposure in monkeys. Archives of Toxicology, 62: 267-273.

S. Schantz, E. Levin, R. bowman, M. Heironimus, and N. Laughlin. 1989. Effects of perinatal PCB exposure on discrimination-reversal learning in monkeys. Neurotoxicology and Teratology, 11: 243-250.

S. Schantz, E. Levin, and R. Bowman. 1991. Long-term neurobehavioral effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys. Environmental Toxicology and Chemistry, 10: 747-756.

APPENDIX E

PREMEETING COMMENTS

United States
Environmental Protection Agency

Technical Review Workshop on
the Reference Dose for Aroclor 1016

Premeeting Comments

Washington, DC
May 24-25, 1994

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Premeeting Comments:

Premeeting Comments

Henry Anderson

Henry A. Anderson, M.D.

Technical Review Workshop on the Reference Dose (RfD) for Aroclor 1016

Pre-Workshop Comments

April 26, 1994

Part I

Element 1:   Selection of Principal Study

While perhaps obvious to some, it would be useful to have a statement on why an Aroclor 1016 RfD is needed. In the concluding paragraph in the "Confidence" section the suggestion is made that it will be of limited utility and the confidence in the RfD is only medium apparently because it is assumed it will be used as a surrogate for assessing the toxicity of mixtures of PCB.

Since the decision was made to evaluate whether it was possible to develop an RfD, the initial decision must be made whether there are sufficient studies of Aroclor 1016 to adequately characterize chronic toxicity and quantitatively derive an RfD. I found the studies summarized sufficient to place bounds around the dose at which toxicity likely occurs. It was the collective results rather than any one study that convinced me that an RfD was appropriate and verifiable. Of the available studies of Aroclor 1016, the series of prospective studies of rhesus monkeys best met the stated criteria for selection of a "principal study" for RfD development. My brief review of the alternatives to these studies as replacements, found the rat, mouse and mink studies to be less desirable. It is a judgement call whether the rhesus monkey studies should be excluded because of QA/QC concerns.

The reasons to utilize these studies need to be more clearly detailed in the beginning of the IRIS documentation. I found the rhesus studies certainly had "warts" but I did not feel they individually or collectively had fatal flaws. Hypothetically, if these studies were excluded, the decision becomes whether the remaining data base is sufficient to support the development of an RfD. I did not find any of the remaining studies as convincing as the rhesus studies - even with their detracting elements. The use of the NOAELs or LOAELs derived from the rat, mouse or mink studies present greater uncertainties in extrapolation which would need to be accounted for, following the RfD development protocol, through application of greater uncertainty factors. It would not be unreasonable to see a combined uncertainty factor of 3,000-10,000 required with such studies. The resulting RfD would be similar or lower than the proposed RfD based on the rhesus studies, and present lower confidence.

Because the rhesus studies have detracting factors which suggest caution in their use, it becomes especially important for the IRIS material to highlight the consistency within the collective studies as well as between Aroclors and perhaps discuss the range of possible RfDs from the various studies. The arguments for why the rhesus monkey is the best species can be found in the document, but it takes some hunting. It would help if the arguments supporting the selection of the "principal study" were clearly stated in the beginning. While I support the committee conclusion, the justification and decision analysis was spread out in the document and required some hunting. The descriptive study data is concisely summarized, but the committee interpretation and judgement decision process is less well delineated.

It would be helpful if a separate paragraph or section would discuss the confounders in the principal studies and the committee decision on the implications to the RfD process more clearly summarized. Additional analyses were performed by the Agency. It should be explained why these were done. The additional analyses (among the reasons appears to be to assess whether decreased gestation length could explain the decreased body weights) need to be better explained and whether the analyses resolved the committee questions. Thus strengthening the study findings and the resulting RfD.

Element 2: Selection of Critical Effects

I support decreased birth weight as an appropriate "critical effect" for the development of the RfD. Decreased birth weight is a well recognized adverse event in animal toxicology. It is not usually considered one of the more subtle toxicologic endpoints. While decreased birth weight is a non-specific adverse effect indicator (compared to specific organ system evaluations such as neuro-behavior, hepatic or immune system function), it has proven a useful animal predictor of potential human risk. Decreased birth weight is also an important indicator of health status in humans. The mean birth weight of the 1 ppm group was 20% less than the controls and the 0.25 ppm group was decreased by 8% (but not statistically significant). This supports a dose-response relationship. But does the 0.25 ppm group represent a NOAEL? It is only a sample size issue that keeps the 8% decrease from being significant. That 0.25 ppm is a NOAEL for this endpoint is supported qualitatively by the 0.025 ppm group that was deleted because of cross-contamination. Despite the contamination, the mean birth weights were the same as the 0.25 ppm group. The impact on birth weight does seem to occur somewhere between the 0.25 ppm and 1.0 ppm dosage.

In any animal or human study the control group is critical to understanding the effect. An alternative to a toxic effect could be that the control births were heavier than normal. If available, it

would be useful to see historic birth weights from this colony. No mention is made of whether the 1 ppm group birth weights were significantly different from the 0.25 ppm study group. If significant, or very close, this would support the interpretation of a toxic effect rather than an artifact possibly due to the control monkeys being better acclimated to the colony, and more mature causing larger babies and the significant group differences.

Greater detail of the re-analysis purpose and results would be helpful. While I think I understand, being more explicit would help IRIS users and strengthen the decision to use these studies.

The discussion accurately reflects the complexity of understanding the pathologic mechanism and interpreting neuro-behavior test results. These can be confusing to understand and difficult to interpret. In any case, I would not interpret these data to provide strong support for a LOAEL at 0.25 ppm Aroclor 1016, the low-birth weight NOAEL.

My interpretation of these rhesus monkey studies is that the investigators did a good job of choosing exposures that likely bracket the true NOAEL and provide greater detail of study design, dose and identification of possible confounders than most studies used to derive RfDs. At this point, I am not convinced that the study results are significantly impacted by the confounders.

Element 3: Selection of Uncertainty Factors

I can support a total uncertainty factor of 100 as being a reasonable choice. The assignment and combination of individual uncertainty factors is a professional judgement. The fact that the choice was a committee determination strengthens the consensus decision. Even with the best of studies and data sets, uncertainty factors less than 100 are seldom used - except for when strong human data is available. Thus, considering the characterized weaknesses in the studies and the lack of multiple studies in the same species, a higher uncertainty factor - perhaps 300 could also be defended (3 for sensitive individuals, 3 for monkey to man, 3 or 10 for Aroclor 1016 specific data limitations, and 3 or 10 for sub-chronic to chronic. My acceptance of the 100 (3,3,3,3) is based on the extensive data on all PCBs and the opinion it is unlikely 1016 would provide any surprises if all the PCB studies had been done using 1016.

An additional reason for not considering the study a "chronic" study (supporting increasing the uncertainty factor) is the observation that the body burden of Aroclor 1016 did not appear to have reached steady-state at the time of conception (doubled between the 4 and 7th month of dosing) and went up at parturition. Although the daily dose of Aroclor 1016 was steady, the tissue levels of PCB appear to have been increasing over the course of the pregnancy. If body burden (tissue concentration) is a significant

contributor to the toxicity, then attributing the effect to the daily dose may underestimate the "chronic" toxicity, if tissue levels have not equilibrated. This issue becomes even more important if the toxic effect is the result of a narrow time period during the early rather than the late pregnancy stages.

Element 4: Weight of Evidence Conclusions

It would be helpful if a "critical issues" section were added. The summary in the "Charge to Reviewers" does a nice job and a similar approach would be helpful in this section. The purpose of this section seems to be twofold. First to concisely summarize the remaining literature, and second, to point out how those studies support specific decisions made in constructing the RfD. Since perhaps the most important information is the NOAEL, LOAEL reports from the studies, it might be useful to have a table listing these. This would be more convenient than having to read all the summaries to get that information.

I would move the portions that are justification for the critical study (such as the similarity of human and monkey metabolism) into the first section. Similarly the discussion of placentation in the "Confidence" section is further support of the study choice and would contribute to the initial section.

I think it would be helpful to include a discussion/description of the Aroclor 1016 tissue levels described in the various studies. While traditional toxicologic descriptions of dose use the mg/kg/day convention, the actual tissue-delivered-dose can provide additional useful information. As mentioned in an earlier section, the issue of achieving equilibrium between dose and circulating/tissue PCB is worth discussing. Does being on the steep slope of accumulation differ from being on a more gradual increase as equilibrium is neared? These tissue data would be especially useful in comparing the animal doses to what is seen in humans.

Part II

My initial recommendation to RfD/RfC work group is Option B. After reviewing the IRIS assessment, the background material sent to the reviewers and the Agency for Toxic Substances and Disease Registry (ATSDR) toxicological profile for the PCBs, my initial impression is that the proposed Aroclor 1016 RfD reasonably reflects the chronic toxicity of the compound. Since scientific research seldom (if ever) provides a truly definitive data-set which uniformly meets every expectation, the differing scientific approaches to addressing the realities of the Aroclor 1016 study deficiencies via the risk assessment process will trigger debate. The background material indicates that the RfD/RfC work group was well

aware of the problematic aspects of the Aroclor 1016 data base. I found their evaluation approach appropriate, if not always clearly enunciated and documented. The studies are well presented, but the decision logic not always clear.

Of course, I may change my recommendation based upon the other reviewers comments and the workshop deliberations. I look forward to a lively discussion.

Douglas Arnold

Premeeting Comments

A. Part I - Selection of Principal Study

The selection of Barsotti's study, wherein female monkeys were fed graded dosages of Aroclor 1016 and their infants subsequently underwent behavioral testing, is the appropriate principal study for the RfD Workshop's consideration based upon my knowledge of the published studies dealing with Aroclor 1016. However, there are several underlying concerns with the determination of the reference dose described in Attachment 1 of the packet received from ERG that require comment.

1. In Part I. A.1. Oral RfD Summary, the matter of how much Aroclor 1016 was consumed by the female monkeys is not clearly evident.

    a) "Conversion Factors: Dams received a total average intake of 4.52 mg/kg (0.25 ppm) or 18.41 mg/kg (1 ppm) throughout the 21.8 month (654 days) dosing period".

    In Barsotti and van Miller, Toxicology 30: 31-44 (1984):
    p.33 "Daily food and Aroclor 1016 consumption was calculated by counting the remaining (feed) pellets and subtracting from the quantity offered".
    p.35 "Feed Analysis: The results of the analysis of the 3 diets for Aroclor 1016 content were 0.700±0.130 (N=12), 0.164±0.031 (N=12) and 0.005±0.001 (N=9) ppm in the 1.0 and 0.25 ppm Aroclor 1016 diets and control chow, respectively".
    p.35 "...the calculated intake of Aroclor 1016 (based on 1.0 ppm PCB) was...".

    Schantz et al Neurotoxicology and Teratology 11:243-250 (1989):
    p.244 "For the PCB-exposed groups, the PCB intake of each animal was estimated by multiplying the number of grams of food consumed by the PCB concentration in the feed".

    It would appear that the Aroclor 1016 consumption was determined solely by counting the number of feed pellets given, determining how many were left, and assuming all of the "missing" pellets were consumed. This is an extremely crude manner in which to determine even qualitative consumption. There appears to be no consideration given to a monkey's habit of lobbing its feed cubes out of its cage and/or "losing" significant amounts of feed in the fecal pans beneath its cage. In short, consumption of Aroclor 1016 may have been overestimated by 1-20%.

Secondly, Barsotti's and van Miller's analysis of the 1 ppm and 0.025 ppm Aroclor 1016 diets was found to be 30% and 34%, respectively, short of the desired concentration.

In summary, Aroclor 1016 consumption appears to have been overestimated, firstly, because the "missing" feed cubes were assumed to have been consumed and, secondly, because the diets do not appear to contain the amount of Aroclor 1016 required by the protocol. Neither of these shortcomings appears to have been addressed when the Conversion Factors were calculated.

b) Further to this point, from Barsotti's thesis:

p.188 "The total intake of Aroclor 1016 by the adult females at the time the infants were weaned was 19.1 ± 4.4 mg/kg for the 1.0 ppm group, 4.6 ± 0.6 mg/kg for the 0.25 ppm group and 0.5 ± 0.1 mg/kg for the 0.025 ppm group (Table 6-2)".

p.208, Table 6-2.

Intake of Aroclor 1016 by Adult Female Rhesus Monkeys	
Level of 1016 in diet (μg/gm)	Intake at Weaning (mg/kg)
1	18.1 ± 3.1
0.25	4.5 ± 0.6
0.025	0.5 ± 0.1

There is an obvious discrepancy between the text values and the Table values.

c) In addition to the discrepancy noted above, Schantz et al (1989), adds further confusion to this matter.

p.247 "Cumulative PCB intake by the 0.25 ppm and 1.0 ppm mothers averaged 4.52 ± 0.56 mg/kg or 7.58 ± 0.28 μg/kg/day and 18.41 ± 3.64 mg/kg or 29.7 ± 2.3 μg/kg/day, respectively".

Regardless of the obvious discrepancies in Aroclor 1016 consumption values between Barsotti's thesis and Schantz et al (1989), the calculation of Aroclor 1016 consumption data to 1/100 of a mg is totally inappropriate given the rather crude manner in which feed consumption was determined/calculated.

2. Some comments pertaining to Part I. A. 2. Principal and Supporting Studies (Oral RfD).

    a) 1st para., 3rd line:

    "Aroclor 1016 is a commercial mixture of polychlorinated biphenyls (PCBs) devoid of chlorinated dibenzofurans (Barsotti and van Miller, 1984)".

Barsotti and van Miller, 1984 p.33:

    "...as was previously reported for American PCBs, (Aroclor 1016) was found to be devoid of chlorinated dibenzofurans [19, J. McKinney personal comm.]".

In the RfD quote, it appears that the Agency has accepted the contention of Barsotti and van Miller that Aroclor 1016, and by implication, the Aroclor 1016 used by Barsotti, does not contain any chlorinated dibenzofurans (CDF). However, the quote from Barsotti and van Miller (1984) is ambiguous. Reference 19 is an article authored by Bowes et al (Nature 256:305-307, 1975) in which Bowes et al did not detect any CDF congeners with 4, 5 or 6 chlorine atoms in the one sample of Aroclor 1016 they analyzed, but Bowes et al did report finding CDF in the other commercial PCBs they tested even though Bowes et al cited previous reports that had not always detected CDF in similar commercial PCB mixtures. Consequently, it appears there was some controversy regarding the presence or absence of CDF in various commercial PCB mixtures when these reports were first published.
The intent of the personal communication from J. McKinney is unclear.

Barsotti's thesis adds further confusion to the issue of whether CDFs were present in the lot of Aroclor 1016 she used:

    "Three of the groups were placed on the diets containing 1.0, 0.25 and 0.025 ppm Aroclor 1016 (<5 ppb dibenzofurans, McKinney, personal communication)." (p.183)

Appendix V of Barsotti's thesis, entitled PCB Analysis, does not indicate that the Aroclor 1016 used in her study was ever analyzed for contaminants. Appendix V only describes the analytical techniques employed in the analysis of the feed, monkey tissues and the breast milk for PCBs. (Barsotti's thesis, Appendix V, pp.231-236.)

In summary, there is no substantive indication either in the Barsotti and van Miller paper or in Barsotti's thesis that the Aroclor 1016 used by Barsotti was ever analyzed

for contaminants, and as a consequence, there appears to be no basis in fact for the contention in the RfD that the Aroclor 1016 used by Barsotti was devoid of CDF.

(Might it be useful to have Dr. Barsotti present at the Workshop so that this and other issues which may be raised could be answered authoritatively?)

b) 1st para., line 5:

"Analysis of the commercial feed used for this study revealed contamination with congeners specific for Aroclor 1248, present in the parts per billion range".

Barsotti's thesis p. 192:

"The finding of higher chlorinated PCBs in control and experimental samples is not surprising since PCBs have been shown to be ubiquitous in the environment. They have been found in commercial monkey chow also (Coleman and Tardiff, 1979). We have shown that the concentration of PCBs in control monkey show (sic) is in the range of 1-50 ppb on the basis of an Aroclor 1248 standard".

The RfD statement clearly states that the monkey chow was contaminated with Aroclor 1248. However, the support for such a statement is unclear.

Barsotti's thesis suggests that the higher chlorinated chromatographic peaks found when she analyzed her diets for PCBs was associated with the Aroclor mixture 1248, but the higher chlorinated peaks were found in both the control and test diets. Both the RfD statement and Barsotti's thesis indicate that the levels of the purported Aroclor 1248 peaks were in the ppb range without further elaboration. However, one is unable to ascertain from either Barsotti's thesis or the RfD as to when the feed may have been contaminated; that is, prior to receipt from the manufacturer or during the pelleting operation.

The analytical methodology used by Coleman and Tardiff as well as the Barsotti analytic methodology (Appendix V) appear to be the same AOAC (Association of Official Analytical Chemists) method. Coleman and Tardiff reported detecting PCBs in 11 of the 12 fed samples they analyzed, but the levels of contamination were less than 10 ppb, having previously indicated their limit of detection for PCBs was 10 ppb. However, Coleman and Tardiff do not comment as to whether the PCBs they detected may have originated from higher or lower chlorinated PCBs congeners.

Consequently, what information does the Agency have in support of its contention that Barsotti's test diets were contaminated with Aroclor 1248 after the feed left Purina's facilities; that is, the contamination occurred during the pelleting process?

As previously indicated (A.1.a), Barsotti and van Miller reported that they analyzed their test diets on 12 different occasions and the control diet on 9 different occasions. Consequently, if Barsotti's diets had been contaminated during the pelleting process with Aroclor 1248, some analytical data should be available to indicate whether the contamination did occur during the pelleting process as well as indicating the duration of the purported contamination. Will the analytical data for Barsotti's diets be made available to the workshop participants?

c) 1st para., line 14:
   "No exposure-related effects on maternal food intake...".

Barsotti's thesis:
p.182 During the monkeys quarantine period the monkeys were "given food and water _ad libitum_...".
p.186 "Prior to breeding there were no changes in food intake...".
p.188 "The animals maintained normal food consumption and body weight..." referring to the adult females.

Barsotti and van Miller (1984):
p.33 "All animals were offered 200 g of chow/day".

The amount of feed offered to the monkeys during the experimental period is ambiguous based solely on the comments in Barsotti's thesis. One might assume _ad libitum_ feeding could easily have been used in view of the manner in which feed consumption was calculated (item A.1.a). However, the Barsotti and van Miller (1984) manuscript states that the monkeys were restricted to 200 gms of feed per day. The troubling aspect of this is that the additional caloric requirements of gestation and lactation may not have been met by the 200 gm daily portion of feed. If the caloric requirements were not met by the 200 gms of feed per day, what effect, if any, might this have upon the infant's birthweight? If there was any effect upon birthweight, might one expect the effect to be dose-related? In view of a possible dietary restriction, might the infant birth weight differences be analagous to the wasting syndrome reported for some halogenated hydrocarbons?

d) 1st para., line 14-16:

"No exposure-related effects on maternal food intake, general appearance, hematology, serum chemistry (SGPT, lipid, and cholesterol analysis) or number of breedings were observed (Barsotti and van Miller, 1984). All monkeys had uncomplicated pregnancies, carried their infants to term and delivered viable offspring".

Barsotti and van Miller (1984):

p.36 "...there were no changes in (maternal) food intake or general appearance of the experimental animals. No significant differences were found in any hematological or serum chemistry values between the 3 groups.

p.36 "After 2.2 ± 0.8, 2.5 ± 1.3 and 1.2 ± 0.4 breeding attempts, all of the females conceived in the 1.0 and 0.25 ppm Aroclor groups and controls, respectively".

Barsotti's thesis p. 186:

"Prior to breeding there were no changes in the food intake, general appearance, hemograms, or serum chemistries. In addition the menstrual cycles of the animals were unmodified as a result of the PCB exposure (Chapter 2). The levels of the circulatory serum estradiol and progesterone during the third and sixth month of the experiment were determined to be similar to those recorded prior to the administration of the diets containing PCBs".

Some (J.A. Moore (1993) IEHR PCBs and Primate Reproduction Meeting August 30-31, 1993; IEHR, Suite 608, 1101 Vermont Avenue, N.W., Washington, D.C., 2005) have suggested that the birth weight reduction observed when PCBs were fed to rhesus monkeys were secondary to maternal toxicity. However, the above statements from Barsotti and van Miller (1984) and Barsotti's thesis clearly indicate that there was no apparent maternal toxicity evident. Consequently, maternal toxicity was not necessary for the marked dose-related decrease in infant birthweight. However, one might muse that the approximate two-fold increase in breeding attempts to attain 100% impregnation in the Aroclor 1016 groups - while not statistically significantly different from the control group, possibly due to the small number of monkeys used in each test group - might be biologically significant and consequently, a suggestive indication of possible maternal toxicity.

e) 1st para., lines 22-34 re:
    Statistical analysis of the infant birthweights

Birthweight vs. gestation length

Birthweight vs. sex of the infants

Birthweight vs. paternal exposure to Aroclor 1248

In view of the amount of birthweight reanalysis done by the Agency, one must ask why no reanalysis was done regarding the distribution of shared paternity described by Schantz et al., 1989; Table 2.

f) 1st para., lines 28-29:

"Males that had sired some infants were exposed to Aroclor 1248...".

Schantz et al, 1989, p.247:

"Beginning in the seventh month of exposure, seven of the females in each group were bred to unexposed males".

Schantz et al. Environmental Toxicology and Chemistry 10:747-756 (1991), p.749:

"The mothers of both PCB-exposed and control subjects were bred with unexposed male breeders".

Levin et al. Archives of Toxicology 62:267-273 (1988), p.269:

"Seven of the females in each group were bred to unexposed males".

What is the Agency's basis for concluding that some of the sires for the Aroclor 1016 females were, in fact, exposed to Aroclor 1248?

g) 1st para., lines 39-40

"Hyperpigmentation was present at birth in the low- and high-dose infants but did not persist once dosing was stopped".

This sentence suggests that the infants were actually dosed. The only exposure to PCBs that the infants received was in utero, via lactation and whatever amount of their dam's diet they may have ingested. Therefore, it might be more correctly indicated that hyperpigmentation did not persist once the infants were weaned and/or placed in their own cage.

h) 1st para., lines 41-42:

"The concentration of Aroclor 1016 in breastmilk was higher than the maternal dose".

This statement might be clarified to indicate whether the comparison is on a wet weight or lipid basis.

3. Comments concerning Part I.A.3. Uncertainty and Modifying Factors
    a) Line 6 "...because of similarities in toxic responses and metabolism of PCBs...".

    Is the data base sufficient that such an assumption is justified, or is it a case of the limited data available indicating that humans and monkeys may metabolize PCBs similarly? The last paragraph of Section I.A.4. and the May 25, 1990, memorandum of Dr. Mike Dourson both indicate that the metabolism of two PCB congeners is similar in monkeys and humans. In my opinion, this is not sufficient evidence to conclude that humans and monkeys metabolize PCBs similarly.

    b) Line 8-9:
    "In addition, the rhesus monkey data are predictive of other changes noted in human studies such as chloracne, ...".

    Chloracne is far from a universal finding when PCBs are fed to monkeys, as per the following references:

    Yoshihara et al (1979) Fukuoka Acta Medica 70:135-171:
    Male and female rhesus monkeys were fed daily dosages of 0.25 or 0.5 mg KC-400/kg/day.
    p.10 "...acneform eruptions, for instance - were not always evident".

    Truelove et al (1982) Archives of Environmental Contamination and Toxicology 11:583-588:
    Aroclor 1254 was fed to pregnant cynomolgous monkeys for approximately 60 days during gestation and for a total of 178 to 207 days after parturition at levels of 100 and 400 µg/kg bw/day.
    No chloracne was observed.

    Tryphonas et al (1984). Toxicology Pathology 12:10-25:
    Aroclors 1248 and 1254 were fed 3 days per week at levels of 4.7 and 11.7 mg/kg bw, respectively to female cynomolgous monkeys for 29-164 days. Death or morbidity generally determined the length of dosing.
    No chloracne was reported.

Tryphonas et al (1986). <u>Toxicologic Pathology</u> 14:1-10:

Aroclor 1254 was fed to female rhesus monkeys for 5 days/week at a level of 200 µg/kg bw/day for 27-28 months.

No chloracne was reported.

Tryphonas et al (1986) <u>Archives of Environmental Contaminantion and Toxicology</u> 15:159-169:

Aroclor 1254 was fed to female cynomolgous monkeys for 5 days/week at a level of 200 µg/kg bw/day for 12-13 months.

No chloracne was reported.

Arnold et al (1990) <u>Food and Chemical Toxicology</u> 28:847-857:

Aroclor 1254 was fed to female cynomolgous (55 weeks) and rhesus (120 weeks) monkeys at a level of 280 µg Aroclor 1254/kg bw/day.

No chloracne was reported.

Arnold et al (1993) <u>Food and Chemical Toxicology</u> 31:799-810:

Aroclor 1254 was fed to female rhesus monkeys at levels of 5, 20, 40, or 80 µg/kg bw/day for more than 3 years.

No chloracne was reported.

c) Line 14:

"As the study duration was considered as somewhat greater than subchronic...".

This appears to be a rather unfortunate phraseology; that is, how can the duration of dosing be somewhat greater than subchronic but less than chronic when the only three choices are acute, subchronic and chronic? Might the author be trying to indicate that the dosing duration for this monkey study greatly exceeded the minimum requirements for a subchronic study with this species but was not sufficient for a chronic study?

4. Comments concerning Part I.A.5. Confidence in the Oral RfD.

a) Para. 1, line 2:

"The initial study was well conducted...".

While the benchmark for a well-conducted study could be an invitation to an open-ended discussion, it is obvious that the level of conduct for this study would have

been much improved if the diets had been analyzed prior to their being fed, and contaminated diets discarded.

b) Para. 1, lines 9-11:
"Although contamination of the control laboratory primate diet...".
See A.2.b regarding the substantiation of the contention as to when and/or if the primate diets were contaminated with Aroclor 1248.

The apparent contamination of the diets with another halogenated compound as well as conceivably with nonAroclor 1016 congeners, without information concerning the apparent duration of the contamination, makes the evaluation/extrapolation of these data more difficult. However, the lack of hyperpigmentation in Barsotti's control infants does indicate that the purported nonAroclor 1016 congeners present in the diet were of very minimal, if any, toxicological significance.

B. Selection of Critical Effects

The reduction in birthweight of the treated dams' infants, in a dose-related manner, is the most significant toxicological effect observed. This finding takes on added significance in view of the fact that it occurred without any apparent maternal toxicity (see A.2.d). However, as pointed out above (A.2.d), it is tempting to speculate as to whether the increased number of matings in the treated groups required to attain 100% impregnation is biologically and toxicologically noteworthy.

C. Selection of Uncertainty Factor

As pointed out in A.4.a, one could argue as to what may constitute a "'well conducted' subchronic study that only defines a LOAEL". If there was consensus by the Workshop members that this study was something less than "well conducted", then consideration of a larger uncertainty factor would be appropriate. However, the seemingly predetermined use of the factors 1, 3 or 10 may truncate such a discussion.

The $UF_s = 3$ may be in need of some revision, if a major consideration for its selection was that "the mothers were probably dosed to 'steady state'". There are very few studies in the published literature that provide any direct insight into this consideration; however, the following studies do provide some information for consideration.

1. Barsotti, et al (1976) Food and Cosmetic Toxicology 14:99-103, p.100:
   "The data obtained from the fat biopsies on the female animals (fed Aroclor 1248 at dietary levels of 2.5 or 5.0 ppm) showed an accumulation of PCB isomers in the

adipose tissue. After a 6 month exposure period, the PCB level in the adipose tissue of the animals receiving 5 ppm attained a plateau, subsequent values varying little with continued consumption of the diet. A similar level was attained in 1 yr by the group given 2.5 ppm PCB".

2. Yoshimura et al. (1981) Fukuoko Acta Medica 72:156-184, p.162:
   "The fact that the blood PCB concentration gradually rose when KC-400 was given (at dietary levels of 0.25 and 0.5 mg/kg) to rhesus and cynomolgous monkeys is the same as in the case of the preliminary study, and in the first administration experiment it reached a maximum at around five months after the commencement of administration".

   While one might construe this statement to suggest a qualitative pharmacokinetic steady state approximating 5 months, subsequent work has demonstrated that the level of PCBs in blood is often related to its lipid content; therefore, blood is not the most appropriate specimen for obtaining data regarding steady state PCB determinations.

3. Arnold et al. (1993) Food and Chemical Toxicology 31:799-810, p.800:
   "...it required approximately 25 months to attain a satisfactory qualitative steady state for 90% of the (Aroclor 1254) treated monkeys (rhesus)". (Dose levels were 5, 20, 40, or 80 µg/kg bw/day.)

D. Weight of Evidence Conclusions

1. Primary Evidence Used for the RfD, item1:
   "The NHP provides conclusive data...".

   The use of the word conclusive seems inappropriate since the second sentence in the introduction of the RfD states "The summaries presented in Sections I and II represent a consensus..."; suggesting a lack of complete harmony amongst the Agency's reviewers.

2. Secondary Evidence, item 2:
   "Chloracne and pigmentation have been observed in both NHP and humans".

Has chloracne been reported when monkeys were exposed to Aroclor 1016? The production of chloracne when monkeys were fed Aroclors was not consistently observed as previously mentioned (A.3.b).

3. Secondary Evidence, item 4:

"In vitro metabolism of several PCB congeners is similar for NHP and humans".

Several is defined by Webster's Ninth New Collegiate Dictionary as "more than two but fewer than many".

Section.I.A.4, last para., of the RfD - "Data exist on the in vitro hepatic metabolism and in vivo metabolic clearance of 2, 2', 3, 3', 6, 6' - hexachlorobiphenyl and 4, 4' - dichlorobiphenyl congeners in humans, monkeys, dogs, and rats (Schnellman et al., 1985). Both of these congeners are present in Aroclor 1016, but hexachlorobiphenyl is only a minor constituent". Since two congeners does not constitute several congeners, was some data inadvertently left out of the RfD summary?

E. Part II. Recommendations

My preceding comments indicate that I do have some difficulty with the current text regarding the analysis/evaluation of the Barsotti - Aroclor 1016 monkey study data. It is my opinion that the limitations regarding the data have not been adequately elaborated. In addition, I also have some concerns about the uncertainty factors. Consequently, if I have to choose an option based on the information provided in the package I received from ERG, I would choose Option C. However, if appropriate information is available at the Workshop, I would be prepared to switch to Option B.

Thomas Burbacher

EPA TECHNICAL WORKSHOP ON THE REFERENCE DOSE
FOR AROCLOR 1016--PREMEETING COMMENTS.

Thomas M. Burbacher

Comments on the selection and use of the four reports listed in attachment 1 as the primary basis for the RFD for Aroclor 1016.

The 4 reports used as the primary basis for the RFD for Aroclor 1016 come from essentially 1 longitudinal study of the reproductive and offspring developmental effects of prenatal and early postnatal Aroclor exposure in nonhuman primates. Three of the reports describe procedures to assess the learning and memory abilities of Aroclor and nonAroclor exposed monkeys or "controls". While these reports describe adequate experimental procedures to test learning and memory in nonhuman primates, the scientific reliability of these reports (and the first report) rest largely on decisions made during the initial phase of this longitudinal study. For it is these early decisions that determine the validity of making comparisons across the Aroclor exposed groups and the "control" group.

The information supplied to me to date is too sketchy to evaluate whether the scientific reliability of these reports is of sufficient quality to be used as the primary basis for the RFD for Aroclor 1016. I do, however, have serious reservations about the quality of the initial phase of the study based on the information provided. I will go into a little detail regarding the basis for my reservations but I would like to mainly provide a series of questions that I believe must be answered before a full evaluation of these reports can be made. It is possible that all of these questions have been answered sufficiently during previous deliberations of this issue. If this is the case, it should not be too much trouble to supply these answers to the panel for this technical review.

When designing a nonhuman primate study to assess the reproductive and offspring developmental effects of a compound, one of the first orders of business is carefully choosing your adult subjects. Ideally, the adult animals that are chosen are of known age and parity, are healthy as determined by past medical records and weights and have a history of no previous invasive studies. Females and males can then be assigned to different exposure and control groups to counterbalance these factors across groups. As anyone who has been involved in nonhuman primate studies knows, however, the ideal situation rarely occurs. When this happens, it is up to the investigator to do the best he or she can to provide groups of adults balanced on as many potentially confounding factors as possible. My first series of questions relates to the characteristics of the adult monkeys at the beginning of the study (when Aroclor exposure began).

-What were the estimated ages of the adult animals in the different groups?
-What were the weights of the adult animals in the different groups?
-What were the colony parities of the 8 females from the general colony who served as controls? I am assuming that the colony parities of the Aroclor exposed monkeys was 0.
-What was the reproductive history of the males used in the study?
-Were any of the adult animals used in previous invasive studies?

EPA TECHNICAL WORKSHOP ON THE REFERENCE DOSE FOR AROCLOR 1016--PREMEETING COMMENTS.

Thomas M. Burbacher

Comments on the selection and use of the four reports listed in attachment 1 as the primary basis for the RFD for Aroclor 1016 (continued).

Another important design consideration for these studies relates to the procedures that are used on adult animals during the entire investigation (including the baseline period). My second series of questions relates to the procedures that were used on adult females prior to and during Aroclor exposure.

-Were the same procedures used on all adult females during the period just prior to initiating the Aroclor and control diets (was there a baseline period)?
-If so, how long was this period and what procedures were used?
-Were the same procedures used on all adult females during the remainder of the study?
-If not, what procedures were differentially used and how?
-Were procedures used to collect data on the daily intake of the Aroclor and control diets?
-If so, are data available concerning the intake of Aroclor during different stages of the study (e.g.., prepregnancy, pregnancy, etc.)?
-Were procedures used to collect data on the weights of adult females during the study
-If so, are data available concerning the weights of females during different stages of the study (e.g.., conception, delivery, etc.)?

There are several other aspects of the initial phase of the study that are not clear from the information provided. The above questions deal with the most important aspects and should be considered a priority for the technical review.

The results of the other studies described in attachment 1 provide little relevant information regarding the association between Aroclor 1016 exposure and decreased birth weight or learning and memory deficits. The studies in mink used an exposure level (2ppm) that showed no effects or a level (20ppm) that was associated with frank maternal and infant toxicity. Reduced weights at this level provide little information regarding possible effects at subtoxic exposures. While the results of the studies of humans exposed to PCB do indicate a birth weight and memory effect, these results cannot be associated directly with Aroclor 1016.

In summary, the information provided thus far indicates that previous reviews of the principal study have concluded that they are of sufficient scientific quality to be used as a basis for the RDF for Aroclor 1016. I would assume that the questions listed above have been answered to the satisfaction of the reviewers during these previous reviews. If this is the case, it should not be too much trouble to get the information needed to respond to these questions for the current technical review.

EPA TECHNICAL WORKSHOP ON THE REFERENCE DOSE FOR AROCLOR 1016--PREMEETING COMMENTS.

Thomas M. Burbacher

Comments on the selection of low birth weight as the critical effect for the Aroclor 1016 RFD, along with information on postnatal neurobehavioral effects..

Birth weight in nonhuman (and human) primates is associated with many maternal, paternal and environmental factors. Well designed studies of reproductive outcome and offspring development attempt to control the possible confounding effects due to these factors in the design of the study or test for these effects in the analysis of the data. For the principal study, the potential effects of maternal age, parity, and weight on decreased birth weight should be considered. Maternal weight gain during pregnancy should be examined for it's possible contribution to decreased birth weight and paternal age and weight effects should be considered. (See comments to first issue for more detail).

The learning effects observed in the Aroclor 1016 offspring at 1.5 years of age are likely to be influenced less by the factors described above than is decreased birth weight. The increase in the number of trials to learn a simple spatial discrimination task in the high dose Aroclor 1016 group may be important evidence of a learning deficit caused by Aroclor exposure. This was the first task in a series of 4 tests for these monkeys. No effects were observed for the subsequent 2 tests and the same group of monkeys performed better than controls on the last test, a color discrimination reversal task, although no details were provided regarding where the effects were observed (on original learning or reversals). Finally, the performance of the Aroclor 1016 infants on a spatial task at 4 to 6 years of age was not different than controls. The learning deficit of the Aroclor 1016 monkeys was, therefore, very specific. Further studies in the area of possible learning deficits associated with Aroclor 1016 exposure are needed to clarify this issue.

In summary, decreased birth weight can be caused by many potentially confounding factors that have to be considered in studies of nonhuman (and human) primates. It is impossible to tell whether these factors were considered in the principal study until more data are reviewed (see comments to first issue). The data presented relating to the learning deficit on the spatial discrimination task may provide a better endpoint for the critical effect for Aroclor 1016. Given the specificity of the effect, however, more studies should be pursued prior to any regulatory actions.

EPA TECHNICAL WORKSHOP ON THE REFERENCE DOSE FOR AROCLOR 1016--PREMEETING COMMENTS.

Thomas M. Burbacher

Comments on the weight of evidence analysis.

The weight of evidence analysis indicates that "the nonhuman primate study provides conclusive data that the reduced birth weight of infants and neurobehavioral effects is consistent with effects observed in other species including the human". The results of studies on mink are used as supportive for the reduced birth weight effect, while studies in rodents are used as supportive evidence for the neurobehavioral effects.

As mentioned earlier, the mink studies used an exposure level (2ppm) that showed no effects or a level (20ppm) that was associated with frank maternal and infant toxicity. Reduced weights at this level provide little information regarding possible effects at subtoxic exposures. The reference to results from rodent studies as supportive for the neurobehavioral effects could not be confirmed with the information provided.

The weight of evidence analysis also indicates that "methodological considerations precluded using the neurobehavioral effects and transient dermal pigmentation as co-critical. It is not obvious why methodological considerations preclude these effects and not the decreased birth weight effect.

Some of the secondary evidence listed provides support for the effects reported in the in primary study. The studies of changes in dopamine may be associated with the learning deficit observed, although effects observed in adult animals may not be particularly relevant to developmental exposure. The chloracne and pigmentation observed in nonhuman and human primates seems similar. The similarity in in vitro metabolism of PCB congeners supports the use of nonhuman primates as an animal model. The decreased birth weight effects in mink are most likely not very relevant due to the doses used.

In summary, it is noted in attachment 1 that the data base available for PCBs is extensive. This may be the case when this data base is compared to most other compounds. However, until the reliability of the primary study is confirmed and additional supportive studies using relevant doses and procedures are performed, I would judge the weight of evidence for the RFD for Aroclor 1016 as weak.

RECOMMENDATIONS

Based on the current information, my preferred option would be C, Revise the Aroclor 1016 RFD value and accompanying analysis in line with peer review recommendations. This recommendation is preferred because more detail of the limitations and associated analysis of the critical study are needed to provide a convincing argument for using the study. The descriptions of supportive studies in the analysis should also include more discussion regarding the limitations of the studies. Finally, this section should also be updated with relevant data published after 1992..

Peter deFur

Selection of Principal Study

The principal study, dissertation research conducted by Dr. Barsotti, and subsequently published in a series of papers in peer-reviewed literature, is appropriate for setting the Rfd for Arochlor 1016. The experimental design, confirmation of administered doses, range of doses, choice of measurement end points and follow-up for as much as four years are strengths of the research. The results of the research from this experiment are consistent with results of related work on other species, although there have been no published corroborations in rhesus monkeys. Factors in the original study, cited in the accompanying materials, leading to questions regarding use of Barsotti and Van Miller et seq., for setting the Rfd, are not sufficient to discount this study.

Selection of Critical Effects

The choice of low birth weight as the critical effect for setting an Rfd is appropriate at this time. The advantages of low birth weight are the comparability to human effects, the power of this measure as a predictor of later adverse effects and the apparent sensitivity of the fetus. This latter is consistent with recent published research on the fetal sensitivity of rats to a chemical (2,3,7,8 TCDD) believed to act through a mode of action common to the PCB's (Mably et al., 1992).

Selection of Uncertainty Factors

The uncertainty factor analysis for Arochlor 1016 is consistent with guidelines and practices used by the Agency. Using an uncertainty less than 10, based on the similarities between the experimental animals and humans, confirmatory results in other species and consistency with other results are appropriate choices for the Agency.

The selection of four categories of uncertainty and exclusion of the fifth category, NOAEL to LOAEL extrapolation, is based on the non-significance of the somewhat reduced birth weight of the experimental group at what was subsequently determined as LOAEL. The Agency may wish to consider further examination of this LOAEL. Considering that the low dose group had a reduced birth weight, albeit not statistically significant, delayed neurobiological and chronic health effects should be evaluated.

Weight of Evidence Conclusions

The primary and secondary evidence support this Rfd for Arochlor 1016. The results are conclusive and consistent both within the study by Barsotti and Van Miller and the research on other species. The difficulty in assessing human responses to known exposure conditions does not weaken the conclusions based on the experimental results.

Recommendation

Confirm the Arochlor Rfd value as in the IRIS entry, with any text modifications resulting from this review.

Mari Golub

Mari S. Golub, Ph.D.

COMMENTS FOR TECHNICAL REVIEW WORKSHOP ON AROCLOR 1016 RfD

INTRODUCTION

After reviewing the materials provided, it is my opinion that the issue that requires most attention from scientific reviewers is selection of endpoint.

Two issues that would profit from clarification based on more information are: the RfD workgroup birthweight analysis; and the rationale for selection of some uncertainty factors.

Diet contamination does not appear to be a major issue from a scientific point of view since internal dose measures are available and the experimental design is not confounded.

PART I. COMMENTS

Selection of principal study

1) Identification of the database:

There are a limited number of studies using Aroclor 1016 dosing. Although the database for PCB[1] mixtures with similar chlorine content is more extensive, there is no indication that chlorine content is an important determinant of noncancer toxicity. In addition for some commercial PCB mixtures with similar chlorine content (Kanechlor 300, Fenclor 42) contamination with PCDF[2] and TCDD[3] may be an issue. Since the RfD is for Aroclor 1016, these studies are not strictly relevant and it seems appropriate to limit the selection of studies to those using Aroclor 1016 dosing.

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[1] polychlorinated biphenyls
[2] polychlorinated dibenzofurans
[3] 2,3,7,8-tetrachloro-dibenzo-dioxin

I am somewhat confused by the statement that the four published reports (designated as the "principal studies") were drawn from the Barsotti doctoral dissertation, since Dr. Barsotti is no included as an author. Perhaps it is meant that the four reports are based on the same groups of animals that were dosed in connection with the Barsotti thesis.

The principal studies used the lowest dosing range; by exclusion they provide the lowest LOAEL/NOAEL. The fact that sensitive endpoints were used in the principal studies prevents any apparent inconsistency with LOAELs and NOAELs from other studies. In particular the mink studies, which also use reproductive endpoints, showed more severe effects at the higher dose range. Similarly, working backward from tissue analysis data, Tilson et al.[4] calculated that RfDs for developmental exposure to environmental PCBs derived from human studies would be somewhat lower (.002-.07 μg/kg/day) than the Aroclor 1016 RfD derived here from nonhuman primate studies.

2)   Quality of study:

Several basic and important features identify this as a good developmental toxicity study: (1) chemical analysis of administered material; (2) measures of internal dose; (3) route of exposure relevant to humans; (4) dose response design; (5) good level of detail in presentation (means, variance and n are presented for all measures); (6) adequate determination of maternal toxicity; (7) statistical group differences with appropriate analysis; (8) publication in peer reviewed journals.

The study did not contain a maternally toxic dose; the Barsotti thesis chapter states that food intake, hematology, clinical chemistry and appearance were not affected in the treated dams. It would be helpful to know how the doses were selected for the study and whether information on the maternally toxic dose was available. However, this is not an important consideration since the purpose of the maternally toxic dose is to preclude false negative findings based on use of an inappropriately low dose range. Since the study did identify an effect, the dose range was *de facto* appropriate.

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[4] Tilson HA, Jacobson JL, Rogan WJ. Polychlorinated biphenyls and the developing nervous system: cross-species comparisons. *Neurotoxicology and Teratoloy* 1990:12;239-248.

A similar point applies to group size. The group size should be selected to provide for sufficient power to detect group differences in the endpoint of concern. Since group differences were detected, the group sizes were *de facto* large enough. Lack of confidence in small groups is not appropriately based on statistical considerations; statistical tests are adjusted for group size. However, evidence for balance in background variables between groups is important when groups are small, and more information on this would be valuable. The description of differences in time in the laboratory is potentially relevant to balance in background factors. However, I am not aware of any information indicating that this factor influences birthweight.

Details are not available in the provided materials to evaluate all the challenges presented in item 3 under Selection of Principal Study. However, the charge to reviewers states that the RfD workgroup has considered these issues and they should be able to respond to requests for information at the workshop. My comments are based on the material available and my experience.

The issue of contamination is not clear to me. The control diet was apparently contaminated with small amounts of both Aroclor 1016 and 1248. Whether the source of contamination was in manufacture or processing in the lab is not known. Since this exposure was common to all groups it would not have biased group comparisons. There were undoubtedly other low level contaminant exposures from drinking water, cage materials etc. as well as from the diet.

No specifics were mentioned concerning lack of conformity with GLP standards. At the time the study was designed, published GLP guidelines would not have been available. However, formal GLP standards were derived from existing practices which should be sufficient to judge the quality of the study.

Selection of critical effect

The three effects noted in the principal studies are reduced birthweight, altered discrimination learning and hyperpigmentation. The reason for not using the discrimination learning and hyperpigmentation endpoints is briefly described as "methodological" (page 5 of Charge to Reviewers).

Both low birthweight and altered discrimination learning occurred at the .028 mg/kg/day dose level. It would seem that both these effects should be mentioned ("co-critical").

The documents mention several reasons why the "behavioral" measures were not used. Page 6 of Charge to Reviewers states that "the behavioral effects were not chosen as critical given the biphasic nature of the response and the lack of statistical power in measuring differences to control". This comment seems to apply to the delayed spatial alternation but not the discrimination reversal tasks. The report of the June 1992 workgroup states that Dr. (Robert) McPhail of HERL evaluated the studies and concluded that the neurological effects observed in the Aroclor 1016 treated monkeys did not differ from the controls. However, no rationale for this conclusion is provided.

A possible factor arguing against use of the discrimination learning effect is the fact that spatial discrimination was impaired while visual discrimination was enhanced. The authors of the Schantz et al. 1989 article provide a convincing biological plausibility argument for the pattern of effects seen in the discrimination reversal tasks. While both tasks involve discrimination, the brain systems involving spatial and visual information processing are clearly distinct. Other behavioral tasks do not assess these same functions. Facilitation of performance of some tasks has been noted in other developmental toxicity syndromes such as those produced by lead and mercury in nonhuman primates as well as in brain lesion syndromes as cited the authors. Abnormalities of most specific endpoints can occur in either direction from the norm. An exception is a global performance measure like IQ or school performance. Testing protocols for nonhuman primates do not include such global measures of behavioral competence but are geared toward specific functions.

Very limited rationale is given for exclusion of the hyperpigmentation endpoint from consideration. This endpoint was apparently identified as critical in connection with the March

1992 meeting of the RfD workgroup. However, the June 1992 meeting designates hyperpigmentation as "not considered adverse". This issue deserves some attention since the NOAEL would be lower. If hyperpigmentation involves a pathological change in skin, rather than a compensatory change, it should be considered an adverse effect in my understanding of the use of the term is risk assessment. I am unclear on the designation of this effect as "clinical", apparently as opposed to "toxicological". The degree of functional impairment at the NOAEL is not particularly relevant to determination of adverse since a continuum of pathology would be expected at higher dose.

Finally, the RfD workgroup analysis of the birthweight data, including gestational age and sex, is not provided. Additional factors valuable in analysis and interpretation of this effect are maternal weight gain and maternal age/size. Maternal age/size, sex and gestational age would be likely to cause group differences in crown-rump as well as weight. Because the products of conception are a fairly small percent of pregnant weight in primates, weight gain is not necessarily tied to fetal weight; however, weight loss could be a relevant factor.

<u>Selection of uncertainty factors</u>

Although the general concept of reduced uncertainty factors and the total UF seem reasonable I am unclear on the specific rationale presented.

1)   Ufa:

I agree that species similarity warrants a reduced safety factor. Since apparently the only reduction consistent with policy is to 3 this is probably appropriate.

2)   Ufs:

It was my understanding that subchronic to chronic uncertainty factors were inappropriate for developmental toxicity studies (USEPA guidelines) because dosing during brief critical periods can be effective. Although this is an RfD and not an RfD_{dt} (as stated in the notes from the November 1992 workgroup meeting) the same logic

should apply. Consideration of bioaccumulation may be relevant but this is not explained. The RfD documents suggest that the monkeys were in steady state for accumulation at the time of conception; thus, longer exposure would be irrelevant. I would appreciate some discussion of chronic vs subchronic developmental toxicity studies and the role of bioaccumulation.

3)   Ufh:

While fetuses *in utero* may be a sensitive population no rationale for this is presented. It would seem that nursing infants would be at greater risk at the same level of environmental exposure due to PCB accumulation in milk. The mention of the use of nonhuman primates as a factor in setting this uncertainty factor is confusing. Shouldn't this be taken into account in Ufa?

I agree that a lower than average safety factor is appropriate for this RfD due to relevant species and sensitive endpoints. I also agree that confidence in the database is enhanced by the large database on dosing with commercial PCB mixtures and on environmental PCB exposure in humans. Although male reproductive and multigeneration studies are lacking, general environmental exposures in domestic and wildlife populations suggest an opportunity to observe such effects. Further, studies with commercial mixtures in laboratory animals have demonstrated lowered sperm counts and lower reproductive organ weights in males at doses in the maternally toxic dose range for exposed females[5].

Weight of evidence conclusions

I agree with the weight of evidence conclusion. Although the database on Aroclor 1016 administration is limited, the database on PCB exposure (secondary evidence) including both humans and animals is consistent with the selection of this study for the RfD, with the

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[5] Golub MS, Donald JM, Reyes JA. Reproductive toxicity of commercial PCB mixtures: LOAELs and NOAELs from animal studies. *Environmental Health Perspectives* 1991:94;245-253.

NOAEL, and with the rationale for safety factors. Although mechanisms for growth retardation and developmental neurotoxicity have not been identified, mechanism studies indicate perturbation of relevant hormonal, metabolic and brain biochemical systems. No studies demonstrating a lack of effect of Aroclor 1016 at these dose levels on these parameters are present in the database.

The problem of dosing with commercial mixtures versus environmental media contamination is a major problem that has been impeding use of scientific information in risk assessment. Hopefully, the exposure assessment and risk characterization components of the risk assessment process will be effective in dealing with this issue in specific cases. At the moment there is no indication that distinct syndromes at distinct dose ranges are produced by exposures via environmental media as versus commercial mixtures. This is illustrated by the literature on mink, where a syndrome initially associated with a contaminated food source was reproduced by administration of commercial mixtures of PCBs.

PART II. RECOMMENDATIONS

The IRIS RfD entry could profitably be strengthened in areas identified by the committee. Most likely, this will involve extending rationale in some areas; the materials provided do not permit the reviewer to reconstruct the basis for the conclusions in the RfD entry. However, it is possible that revision of the RfD will be warranted because conclusions are not backed up by a strong rationale. This may be the case for selection of endpoint. Thus, my choice of options would be either A or C depending on the additional information supplied by RfD workgroup resource persons at the meeting, particularly on the issue of selection of endpoint. However, given the extensive previous internal and external review and public comment on the RfD, I would anticipate that A would be the more likely recommendation at the conclusion of the peer review.

Rolf Hartung

<u>Comments on the Principal Study</u>

The most disconcerting fact about the reference dose (RfD) for Aroclor 1016 (A-1016) is that it is based upon a single group of rhesus monkeys (8 animals per dose level) which received 0.25 or 1.0 ppm of A-1016 in the diet - 8 more animals served as control. Effects were measured in the offspring that were produced during the 22 month exposure period. Basic questions which were not addressed in the RfD document are: 1.- what was the food intake and 2.- what where the body weights of the pregnant rhesus monkeys at the various exposure levels throughout the exposure period?

The 16 exposed monkeys were acquired in 1977, while the 8 control animals were acquired 4 years earlier in 1973. Aside from similarity in breeding success, there is no assurance of similarities in age, body weight, condition, or even whether these two groups originated from the same genetic stock. The appropriateness of the controls is in question, especially when one is trying to assess subtle quantitative differences. If the ages or the genetic stock of the control and exposed monkeys were different, then the differences in a non-specific response, such as birth weights, between exposed and control groups could in large part be due to such differences, rather than being due to exposures to A-1016. This potential compromise of the data may even extend to learning and behavioral differences.

The current write-up of the RfD does not assess all of the factors cited above. In my mind there are unresolved questions regarding the suitability of the principal study and the critical effects that were chosen for the RfD. Furthermore, questions concerning the reliability of the administered dose must be settled.

<u>Selection of Critical Effects</u>

Bodyweights and birthweights are highly non-specific responses, which are not by themselves characteristic of a toxic insult, as an elevated serum transaminase level or histopathologic change might be. This is not meant to assert that bodyweights or birthweights cannot be used as critical end-points, but it does mean that it is very important to assess the influences of potential confounding factors. This has not been done effectively.

The fact that reduced weights were reported in other studies, albeit at much higher doses, is not very corroberative in this case. The reason for this is that reduced weights occur at some dose level in almost any study.

<u>Uncertainty Factors</u>

The selected uncertainty factors are dependent upon the selection of the critical effect and upon the relevant principal study. If that selection is in question and if other studies or other effects need to be selected,

then the uncertainty factors are bound to change as a consequence.

Weight of the Evidence

The Aulerich and Ringer (1977) study in mink suggests an NOEL (not an NOAEL) of 0.4 mg/kg/day or higher, because this was the highest dose tested and therefore this is a free-standing NOEL.

It is suggested that the number of mink tested in the Bleavins, *et al.* (1980) was insufficient, even though the number of mink tested is higher than the number of rhesus monkeys that were tested in the principal study. We are obviously not very consistent here!

The results of the mink studies are very interesting, but is this the appropriate species to consider? Is the reproductive physiology of the Mustelidae, with its many examples of delayed implantation and development, an appropriate model for assessing the reproductive physiology of humans? [R.K. Enders (1952). *Reproduction in the mink (Mustela vison)*. Proc. Am. Philosophical Soc. 96(6):691-755.]. Furthermore, the mink has been shown to be much more sensitive to tetrachlorobiphenyls than the rat, and to develop apparently species-specific responses in the form of a necrotizing enteritis [D.M. Gilette, R.D. Corey, L.J. Lowenstine and L.R. Shull (1987). *Comparative Toxicology of Tetrachlorobiphenyls in Mink and Rats*. Fund. Appl. Toxicol. 8:15-22].

Recommendations

My preferred option is C for the reasons indicated in my comments.

Nancy Kim

Part I. Selection of the Principal Study

Given the data base for Aroclor 1016, the choice of principal and supporting studies on which to base the RfD is reasonable. The issues that have been raised about impurities and handling of animals are legitimate, but the dose response in birth weight helps to off-set the importance of these questions.

Although human studies are used to support the finding of reduced birthweight in rhesus monkeys, one problem with the studies is the apparent inconsistency between the severity of the effect in humans and monkeys, given similar PCB serum levels. For example, Taylor et al. estimated that an increase in a woman's PCB serum level from 10 to 20 parts per billion (ppb) would be associated with a decrease in birthweight of 23 grams (g), or about 0.7 percent given an expected birthweight of 3,300 g. In the 1016 study, an increase in the mean PCB serum level in rhesus monkeys from 12 ppb (dose level = 0.007 milligrams per kilogram per day--mg/kg/day) to 27 ppb (dose level = 0.028 mg/kg/day) was associated with a reduction in birthweight from 486 g to 421 g, a 13 percent decrease. Even though the studies differ in many ways and each has its own limitations, such a disparity in findings raises concerns about possible differences in sensitivity between the two species and suggests additional work on interspecies sensitivities to PCBs is warranted.

Selection of Critical Effects

The selection of low birth weight as the critical effect for Aroclor 1016 is appropriate given the present data base. The study shows a clear cut effect for the endpoint, a no-observed effect level (NOEL) and a dose response. The possibility of neurobehavioral effects raises concern, but given the questions associated with those effects and the studies, it is prudent to view those results with some uncertainty. The Agency may want to examine these findings in conjunction with the experts who participated in developing the neurotoxicity risk assessment guidelines.

The Agency needs to state clearly why it did not use the neurobehavioral effects in determining the NOEL/lowest-observed effect level (LOEL). The studies are discussed and the IRIS write-up leaves the impression that PCBs probably did cause the observed neurobehavioral effects. If so, the Agency should state clearly why these data weren't used

and what additional data are required before the Agency would use neurobehavioral data to develop an RfD.

Hyperpigmentation was mentioned as being present at birth in both the high and low dose infants and it did not persist once dosing stopped. The Agency determined that this was not a critical adverse effect and the impression is given that at least part of the basis was that it was transitory. Given that a RfD is meant to be a lifetime exposure level without an appreciable risk of adverse effects, one could argue that the hyperpigmentation is an adverse effect, would not be transitory under continuous exposure conditions and should be considered in the RfD development. Another possible concern is whether a relationship exists between hyperpigmentation and melanoma, a cancer linked to PCBs in a recent epidemiological study of capacitor workers.

Selection of Uncertainty Factors

The magnitude of the uncertainty factor seems appropriate. However, the Agency derives the overall uncertainty factor by considering and developing an individual factor for five areas of uncertainty, and then multiplying them to obtain the overall uncertainty factor. In the case of Aroclor 1016, the four factors of three lead to an uncertainty factor of one hundred. One inherent problem with the Agency's generic process for determining uncertainty factors is that it requires the "pigeon-holing" of factors that cross the areas of uncertainty. In the charge of reviewers, the use of a non-human primate (NHP) is given as the reason for the factor of three for both the uncertainty factor for interspecies extrapolation (UF_A) and the uncertainty factor for intraspecies extrapolation (UF_H). Its use on UF_A is appropriate, but its use under UF_H seems inappropriate. Moreover, its use implies using an uncertainty factor of three for UF_H for every developmental/reproductive toxicant when the chronic reference dose is based on effects caused by transplacental exposure. Thus, the process for choosing an uncertainty factor for a specific toxicant may lead to some generic policies that are unintended. The selection of the overall uncertainty factor should be provided in a weight of the evidence approach, without using mathematical formulae. The basis for setting uncertainty factors should be compared for Aroclor 1016 and Aroclor 1254.

Weight of the Evidence Approach

The weight of the evidence approach described in the charge to the reviewers and in the IRIS write-up are similar, but both are somewhat paradoxical regarding the weight of the evidence given to the neurobehavioral studies. The charge to reviewers states (page 5, item 1 under Primary Evidence Used for the RfD) that, "The NHP study provides conclusive data that...neurobehavioral effects is consistent with effects observed in other species, including humans." However, on page 6 (item 2) it states that, "The behavioral effects were not chosen as critical given the biphasic nature of the response and the lack of statistical power in measuring differences to controls."

Part II. Recommendations

I recommend option B. The RfD value is reasonable, given the existing data. The text should be rewritten to give a clear understanding of the Agency's rationale for dismissing the possible neurobehavioral and dermal effects as basis for a RfD.

Because IRIS is used by many people who are not familiar with the toxicological data base for PCBs, the write-up should be careful to give accurate impressions. For example, the eighth paragraph under additional studies has the sentence, "due to uncertainties regarding actual sources of PCB exposure, and other confounding factors and study limitations, the decreases in human birth weight cannot be solely attributed to PCBs, particularly specific PCB mixtures." Having the word 'solely' in the sentence would lead someone unfamiliar with the Yusho and Yu-Cheng incidents to assume that the effects were primarily due to PCBs. In addition, the last sentence of this paragraph overstates the weight of evidence that PCBs caused birthweight reductions in humans.

The basis behind the selection of the uncertainty factor should be rewritten.

Additional technical issues that could be developed in the future include comparing reproductive/developmental effects with serum levels across species and developing toxicity equivalency factors for reproductive/developmental effects for individual congeners.

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Ralph Kodell

Review of RfD for Aroclor 1016   Ralph L. Kodell

Part I.   Comments.

Selection of Principal Study

The principal study has several weaknesses, but I do not think that they are serious enough to disqualify the data.

I agree that the low level contamination of the diet with Aroclor 1048 does not compromise the comparisons of dosed groups to controls, since the contamination was consistent across treatment groups, and its concentration was orders of magnitude less than the levels of Aroclor 1016.

The small treatment group sizes are not that much of a concern, particularly since there *was* sufficient statistical power to detect a difference between the high dose and control. For monkeys, eight animals per group is a fairly high number.

Exclusion of the lowest dose group from published reports because of PBB contamination does not appear to compromise the study.

My main concern, in terms of study design, is the fact that the animals were not assigned randomly to treatment groups. Apparently, the animals in the Aroclor 1016 groups all came from a homogeneous group, but the controls came from a different source. Presumably, the control animals were older, having been obtained approximately 4 years before the treated animals. Since there is a confounding of animal source and Aroclor treatment, it's not possible to determine either 1) whether the observed effect in the high dose group was due to Aroclor 1016 or to a difference in animals, or 2) whether the failure to detect an effect at the low dose was due to there being no real Aroclor effect, or due to a difference in animals that masked the Aroclor effect. Since the high dose group and low dose group differed significantly from one another (not shown, but true), it is reasonable to attribute the effect seen at the high dose to Aroclor 1016.   There is less certainty associated with identifying the low dose as a NOAEL for Aroclor 1016, although it might be reasonable to do so.

Selection of Critical Effects

Assuming that the principal study is acceptable, then average birth weight is a relevant response on which to base the RfD. It appears that the neurobehavioral data support the birth weight data, in that an effect of Aroclor was detected at the high dose but not the low dose. The cited human data are consistent with the reduced birth weight attributed to Aroclor 1016 in the principal study.

None of the additional studies appears to offer a better basis for setting an RfD. Certainly, none of them establishes a lower NOAEL than that in the principal study.

Selection of Uncertainty Factors

The overall uncertainty factor of 100 seems appropriate, although I would partition it out differently.

UF_A: A factor of 3 is justified by the documented similarities between monkeys and humans. I think 10 would be unnecessarily conservative.

UF_H: I agree that transplacentally exposed infants are a sensitive subpopulation. Thus, it seems that a factor of 1 could be justified, and that the present factor of 3 is conservative.

UF_D: I believe that the questions raised regarding the principal study, along with the noted lack of data on other types of reproductive studies, definitely demand a factor of at least 3. Since I plan to suggest a modifying factor, I would leave UF_D at the present value of 3.

UF_S: I'm not sure what is considered an appropriate duration for a chronic study in monkeys. But I feel that a factor of 3, as used, is adequate to cover the uncertainty.

Assuming that the observed effect on birth weight is truly due to Aroclor 1016, then the small sample size of the principal study and the confounding of Aroclor treatment with animal source become more of a concern, since the mean birth weight at the NOAEL was

numerically lower than the control, although not statistically so. If a benchmark-dose approach were to be used, it is possible that a value lower than the established NOAEL would be identified as the benchmark dose. I believe that there is sufficient uncertainty regarding the design and conduct of the study to warrant a Modifying Factor of 3.

Thus, although I would lower UF_H from 3 to 1 and add a modifying factor of 3, I would still come out with the same overall uncertainty factor of approximately 100.

Weight of Evidence Conclusions

I believe that the primary and secondary evidence are well summarized in Attachment 1. I think that a *medium* level of confidence in the RfD for Aroclor 1016 is about right for the reasons stated in Attachment 1.

Part II Recommendations.

At the present time, I would choose **Option B**. I think that the RfD for Aroclor 1016 should be confirmed as presented in the IRIS entry, but that the text should be revised to include a broader discussion of data limitations and related uncertainties.

In spite of the limitations of the principal study on which the RfD for Aroclor is based, I doubt that a better, more appropriate data set can be identified as the basis for setting a revised RfD. Also, given the chosen principal study, I think that birth weight is the most appropriate endpoint on which to base the RfD. Although I think the principal study does provide the best data available, I believe that it has sufficient limitations to warrant applying a modifying factor of 3. However, I would offset this additional factor of 3 by reducing the UF_S from 3 to 1, since I consider transplacentally treated infants to represent a sensitive subpopulation.

Philip Leber

COMMENTS ON REVIEW OF RfD FOR Arochlor 1016

A. Philip Leber, PhD

May 2, 1994

I. Suitability of Proposed Study (Barsotti) as Basis of RfD

A. Real concerns exist with regards to the adequacy of the published information on this study. In general, sufficient detail is not given in a number of areas to substantiate cause-and-effect findings for Arochlor 1016 and adverse findings in rhesus monkeys. The bases for this conclusion are presented below:

1. Characterization of the test chemical/diet.
Information presented is very sketchy, and inadequate to provide the reviewer understanding of what test animals were exposed to during study. For example, the material was indicated to be Arochlor 1016 without a detailed description of the percentages of not only how many chlorines were bonded to the diphenyl moiety but which isomers were found. This is profoundly important as it is now known that certain PCBs have TCDD-like activity (some was seen in this study) whereas others are devoid of this toxic activity.

Secondly, the papers indicate that the test material was devoid of "dibenzofurans", citing personal communications with McKinney. No mention was made on whether chlorinated dibenzodioxins were looked for or found. Additionally, the possible presence of these contaminants is such an important issue that a detailed report on analytical efforts to evaluate the test material not only for trace amounts of the dibenzo-furans and -dioxins but also to provide a full qualitative and quantitative accounting of the makeup of the test substance.

The basis for the need for more comprehensive data on test material identity is multitudinous. First, if a NOEL or LOEL is to be derived from this

type of study, what conclusions can be reached in terms of what caused the various toxicities? For instance, if the material was 90% component A, 8% B, and 1% C, what is the causative principle for each toxic response, A, B, or C? This is a pertinent issue in this study since only certain (unknown) constituents or metabolites of the test substance appeared in maternal or offspring fatty tissues or maternal milk.

Secondly, a perhaps more importantly, how can the Agency regulate PCBs on a basis other than on individual isomer basis? Clearly, if component C accounts for the toxicity of the test mixture, RfDs/RfCs need to be established for this substance and not the mixture as a whole. In the scenario where A and B do not account for significant toxicity, how would having an RfD for the mixture rationally address health effects, particularly since the ratios of A, B, and C are likely going to change once introduced into the environment.

Thirdly, there is evidence that monkeys were inadvertently exposed to PBBs, Arochlor 1248, or unspecified PCBs. While it has been estimated that the exposures were quite low, it raises a question of how thoroughly the diets were examined to ascertain the exclusion of halogenated dibenzo-furans or -dioxins. The adverse findings in this study suggest a potent activity on the part of the test substance, and since it is known that there are potential contaminants of PCBs/PBBs that possess the qualitative quantitative attributes which could lead to the findings observed in the study, any conclusions which attribute cause-and-effect are tenuous until test diets are unambiguously shown to be devoid of these contaminants.

It was stated that women which were exposed to PCBs gave birth to lower-weight offspring, and that this information is consistent with the Barsotti study results. Again, however, it is acknowledged that the chemical etiology in the human episodes is not clear, and therefore, it is tenuous to be discussing an RfD for the 1016 mixture where only one component may be important toxicologically, both for lab animals and humans.

It is concluded that because of incomplete analytical information on the test material, there are tasks which cannot be performed with any scientific certainty. The first is a toxicological issue which involves the chemical causation in the Barsotti study. Are the adverse effects reported related to a PCB isomer, and if so, which one(s) and at what concentrations, or are they related to perhaps to a non-PCB (e.g., chlorinated dibenzodioxin). Secondly, when NOELs and LOELs are proposed from this study, and an RfD is derived for Arachlor 1016, how can the Agency apply this reference dose to environmental media which will be assessed as containing certain PCB isomers. For example, if the Agency finds 2,3,3',4 tetrachlorodiphenyl chemical in stream sediments but only has RfDs for Arochlors (mixtures), how can risks be assessed in this situation?

2. Lack of adequate information on test animals.

The "thesis" of Barsotti did not include certain information on the control female rhesus monkeys in the study. Because these animals are outbred, it is important to characterize the test groups and controls as fully as possible, and to ensure random assignments of test animals. There is no evidence that animal randomizations (essential for minimizing bias in this type of study) were performed, either for maternal or paternal monkeys. Additionally, initial and weaning body weights for dams were given only for PCB test groups but not controls. Since offspring weights can be related to maternal body weight (BW), inclusion of these data are important if deficits in the offspring are being used as a criterion for adverse effects. Descriptions of procedures on health status that are normally carried out during quarantine periods, breeding and dosing schedules, male and female mating histories and design of pairings of rhesus monkeys, were also absent.

Although birth weight deficits can be a legitimate adverse effect for basing risk assessments, the 6 and 15% decreases in this parameter appear to be very "soft" findings in this study because of the apparent heterogeneity of the maternal population (BWs, lengths of time within the colony, age?, other unknowns), and the low number of animals in the test

groups. In addition, although the infants continued to be exposed via maternal milk to Arochlor, 17-week data indicate that the BW difference between controls and high-dose rhesus were virtually eliminated. In other words, it may be expected that this gap would have widened as infants' exposures continued.

3. Uncertainty Factors

Interspecies (UFa)

The rhesus is very sensitive to the effects of PCB products as is the human. However, before assuming that a value of 3 is needed which implies that the human is more sensitive, a comparison between thresholds or sensitivities for effects such as chloracne or neurological effects may indicate that a value of 1 is appropriate.

Intraspecies (UFh)

It's true that transplacentally-exposed infants appear to be a sensitive population. A value of 3 is appropriate for the uncertainty factor.

Overall Database (UFd)

Since developmental endpoints appear to represent the most sensitive endpoint for exposure to Arochlor 1016 and this has been evaluated in a species which has a susceptibility similar to that of humans, a factor of 3 is quite adequate for this category.

Lack of Chronic Data (UFs)

The Agency acknowledges that animals in the Barsotti study were exposed to "steady state" levels, and that consistent with human findings, developmental effects are likely the most sensitive effects from exposure to these agents with chronic testing not likely leading to a lower NOAEL. It is proposed that given the partial accounting for the developmental endpoint in the UFd factor of 3 presented above, a value of 1 appears to

be adequate. No other toxicities were noted other than those
considered to be "developmental" in the 22 month study.

Because of the evidence that rhesus are a very sensitive species, and
that the developmental endpoint appears to be represent a most
sensitive finding following exposures to PCB products, an overall UF of 10
applied to the NOAEL for the study appears to be scientifically justified.

4. Weight of Evidence

Study

It appears that the exposure of pregnant monkeys to a test diet which
contained chemicals including those in Arochlor 1016 led to adverse
effects in offspring. However, in the determination of an RfD for Arochlor
1016 per se, the confidence in this study is considered low. This is based in
part upon the fact that, qualitatively and quantitatively, there are many
uncertainties in the compositions of the test material and diets. In
addition, as it was stated in communications for this review, the 1016 is
expected to be less toxic than the more highly chlorinated products.
However, this study demonstrated very high potency for 1016, raising the
question whether effects are related to the main components (PCB
isomers) or trace contaminants.

Data Base

Unquestionably, adverse effects have been observed in many species
including humans as a result of exposure to PCB products which may
include certain highly potent reaction products that interact at dietary
concentration in the ppb range with the TCDD receptor to cause an array
of toxicities. It is also known that certain PCB isomers have greater
toxicity due to certain conformational features. While data exist which
support the findings in the Barsotti study, conclusions addressing chemical
cause-and-effect also exist in this literature. Again, in the determination
of an RfD for Arochlor 1016 per se, the confidence in this information is
considered low.

RfD

Overall, the confidence in the RfD must be considered low.

5. Recommendation for Action.

Option D

My suggestion is to consider RfDs for components of PCB products according to findings for individual components. While this may at first be considered to be tedious (given the multitude of isomers), in vitro and modern computer SAR techniques are approaching adequacy for ranking these discrete chemicals in terms of toxic actions. Using databases from "purer" PCBs will be needed. Such an approach avoids the substantial problems posed by (a) trying to develop RfDs when relying solely on data derived on chemical mixtures such as that developed by Barsotti, and (b) applying RfD values to PCB contamination scenarios where the chemical analytical findings cannot be related to discrete products (e.g., Arochlor 1016). While there may be occasions where only one source of contamination is likely (as is suggested), the makeup of the contaminated media will be constantly shifting. Attempted application of an RfD derived from a "mixture" tox study to an environmental media "mixture" condition appears to be a scientifically untenable exercise.

John Moore

Part I. Comments

SELECTION OF THE PRINCIPAL STUDY

The reference dose document clearly states that the critical effect selected was reduced birth weight. Thus the primary data source is Dr. Barsotti's doctoral thesis, a secondary source is the Barsotti and Van Miller (1984) article in *Toxicology* that drew from her thesis data. The Schantz et al. (1989) (1991) and Levin et al. (1988) papers do not reflect data from the Barsotti thesis. Rather, they reflect studies performed on some offspring from the Barsotti research that was performed at later time periods. Statement 1 on page 2 of the Charge to Reviewers should be corrected to reflect these facts.

The Charge To Reviewers states that the principal study must be of "sufficient quality." While the term is not further defined, sufficient quality must be judged in the context of its proposed use in a risk assessment. In this instance there is a need to 1) critically assess the certainty that Aroclor 1016 does affect infant primate birth weight, and 2) review the logic applied in quantitatively extrapolating from experimental data to establish a value relevant to human health. Several issues merit review that are summarized below.

COMPARABILITY OF CONTROL AND TREATMENT GROUPS:

Issue: Birth weight is known to be affected by maternal age, parity, and nutritional status, factors that were not controlled in allocating monkeys to either control or treatment groups or within treatment groups.

Discussion: All primates were identified as feral animals with no reference as to geographic habitat or country of origin. Dr. Barsotti was quite clear in stating that the control monkeys were a group that had been in captivity since 1973 and that the animals used in the Aroclor 1016 dose groups were received from shipments in 1976 (7/21/76 and 10/20/76; Table 2-2, page 56 of thesis).

It was stated that some of the animals appeared younger than others which was clarified by irregularities in their menstrual cycles (thesis page 185). It was further stated that because of this

immaturity nine months were required before regular menstrual cycles were established. No such issues were raised in a discussion of the control monkeys.

Concern: An Aroclor 1016 "effect" was determined by a comparison of birth weights from treated and control groups. The treated and control groups had a number of important differences other than exposure to Aroclor 1016. Treated and control monkeys differed by several years as to length of time they were in a closely controlled environment and fed a standard laboratory diet. Given the three-year difference in dates of receipt, it is reasonable to assume that the controls were older than the treated monkeys. It is quite probable that there was a difference in reproductive parity between control and treated monkeys. It is known that there were significant age differences within the group of primates that were assigned to one of three treatment groups; there is no indication that allocation within treatment groups considered this factor. Small group size could magnify any effect due to this factor.

CHEMICAL CONTAMINATION

Issue: General procedures in use at the time were not sufficiently stringent to preclude cross contamination between studies with PCBs, PBBs or TCDD. There is substantial reason to believe such events occurred in the Aroclor 1016 study which call into question any judgment that there was a clear causal relationship between exposure to the test material and reduced birth weight.

Background: Barsotti acknowledged that the Aroclor 1016 group that received 0.025 ug/gm in the diet had, through procedural error, received a PBB diet for an unknown period of time. This error was detected when unexpected peaks were observed in gas chromatograms of tissues although no chromatograms were found in the thesis to verify the point.

Barsotti acknowledged in her thesis that the commercial diet used at the primate center contained low levels (0-50 ppb) of PCB which they characterized as being similar to Aroclor 1248.

The toxic effects of Aroclor 1248 were studied at Wisconsin, indeed these studies constituted the major portion of the Barsotti thesis. Aroclor 1248, which contains a much greater proportion of

contaminant, attain levels in control animals equivalent to the mean levels observed in 5 mothers at parturition who had consumed a diet containing 0.025 ug/kg for over a year (Table 6-1, page 207 of thesis). The control diet was reported to contain Aroclor 1016 only at the limit of detection (0.005 ug/gm).

Table 6-4 (thesis page 210) lists two control infants as having Aroclor 1016 skin levels (fat basis) of 1.0 and 2.07 ug/gm which is well within the range reported for the 7 infants born of mothers who had been fed a diet that contained 0.25 ppm Aroclor 1016 for almost a year. This does not track at all with what is known about the pharmacokinetics of these materials. It does raise questions about what exactly was the maternal exposure.

SELECTION OF CRITICAL EFFECTS

For all of the reasons outlined in the previous section, selection of lower birth weight can not be considered a critical effect with even a small degree of confidence.

Section 7 of the material provided to us contains material associated with a 06/23/92 RfD/RfC Work Group meeting. In that document it is stated that HERL/NTD reviewed the neurobehavioral data, concluded that because the dosed groups did not differ significantly from the controls neurotoxicity should not be considered a critical effect. It further stated that Dr. McPhail had reviewed the Schantz et al. (1989) (1991) and Levin et al. (1988) papers in reaching the above conclusion.

While it would have been of value to have included Dr. McPhail's written review in the material provided to us, it must be assumed that he represents the scientist with the best qualifications to critically review such data. It was noted that despite the extensive number of meetings held to review this data, the individuals who actually reviewed the material and their qualifications and experience were never identified; in fact those who attended meetings apparently were not recorded.

SELECTION OF UNCERTAINTY FACTORS

It is not apparent that an uncertainty factor needs to be applied based on the logic that this is a less than a chronic study. A study that has a dose regime that spans almost an entire year would seem to qualify as chronic especially since there was data presented that indicated steady state tissue levels were reached after about four months of dietary exposure.

WEIGHT OF EVIDENCE CONCLUSION

Primary evidence: The material provided disputes the statement on page 5 of the Charge to Reviewers, i.e., there was no conclusive evidence of neurobehavioral effect. Further, there are no human data that identify Aroclor 1016 as having neurobehavioral effect. There is little certainty of judgment associated with extrapolating results from higher chlorinated PCBs to results observed with lower mixtures.

Part II. Recommendation

Option D. Withdraw the RfD for Aroclor 1016 and state that there is insufficient data to permit the establishment of such a value for this PCB mixture.

James Olson

James R. Olson
5/2/94

Comments Regarding the RfD for Aroclor 1016

Aroclor 1016 is a commercial mixture of PCBs containing about 41% chlorine by weight. The Drinking Water Criteria Document for PCBs (US EPA, 1989) summarized the relative composition of Aroclor 1016 as containing 1% mono Cl, 20% di Cl, 57% tri Cl, 21% tetra Cl, and 1% penta Cl biphenyls. A comprehensive list of individual congeners was not included in this document. PCB Congeners representative of Aroclor 1016 have been detected in finished drinking water obtained from the Hudson River and samples from well water taken during the National Organic Monitoring Survey (US EPA, 1989). Since PCBs are always present in biological and environmental specimens as a complex mixture of individual congeners, with varying pharmacokinetics and environmental persistence, it is most of the time not possible to specify PCB levels in terms of a specific commercial formulation, such as Aroclor 1016. While this is a major limitation of developing a RfD for Aroclor 1016, there are few alternatives based on the available scientific data.

-It would be useful to define the chemical composition of Aroclor 1016 in greater detail in the RfD Summary.

-The RfD Summary should also contain some discussion of the limitations associated with giving a RfD in terms of a complex commercial formulation.

Selection of Principal Study

The principal study used for the Aroclor 1016 RfD was published as four periodic reports on a single group of rhesus monkey mothers and their offspring, including follow-up data for up to four years after birth (Barsotti and van Miller, 1984; Levin et al., 1988; Schantz et al., 1989; Schantz et al., 1991).

The following comments are related to problems associated with the above studies.

Selection of Controls:

Eight animals purchased in 1973 were used as controls, while 16 animals acquired in 1977 served as experimentals (Barsotti and van Miller, 1984). All animals were purchased from the same supplier. There remains a question as to whether the Aroclor and control exposures occurred during the same time. If the entire study was conducted at the same time, the control animals had 3-4 years longer to acclimate to the laboratory conditions. Data on the age and body weight of the control animals was also not provided. This is of concern since body weight data on the Aroclor exposed adult female monkeys was given prior to exposure and at weaning (Table 6-8, p 214 of Barsotti's dissertation). Birth weight could vary with the age and body weight of the animal. In addition, no data were given on the reproductive history of the animals. Birth weight could also vary with the number of prior pregnancies which resulted in live births. Perhaps it would be possible to get answers to these questions.

Although some questions remain regarding the control group, the data support a dose related decrease in the birth weight of the offspring with exposure to Aroclor 1016. A significant decrease was reported between the control and high dose group. Furthermore, there was a significant decrease in birth weight between the high (1 ppm in food) and low (0.25 ppm) exposure groups ($p < 0.001$, unpaired t-test). Aroclor 1016 concentrations in the skin of the infants at birth further confirms the dose related transplacental exposure of the infants to Aroclor 1016.

Dosing and Contamination Issues:

In the original study, a 0.025ppm 1016 in the food exposure group was also included. Published data from the study did not include this group due to PBB contamination in this diet. This problem was identified by the authors because they conducted extensive analysis of animal tissues and diet during the study and discovered the PBB contamination.

The ubiquitous presence of PCBs appears to be responsible for the monkey chow containing from 1-50 ppb PCBs based on an Aroclor 1248 standard. The higher chlorinated PCBs were found in control and experimental diets and tissues.

Dosing and contamination issues are of some concern but appear to be largely due to the ubiquitous low level persistence of PCBs in the environment. The strength of the study is still the dose related decrease in birth weight which is related to tissue levels of Aroclor 1016 in the infants. Another strength of the study is the extensive chemical analysis of the diet and animal tissues, confirming exposure to Aroclor 1016.

Although there are problems associated with these studies, these factors do not appear at this time to disqualify use of these reports as the principal study.

Selection of Critical Effects

Reduced birth weight in the rhesus monkeys in the principal study was identified as the critical effect for the RfD. As stated above, there was not only a significant decrease in birth weight in the high exposure (1ppm) group relative to the control, but also between the high exposure (1ppm) and low exposure (0.25ppm) group. The dose related decease in birth weight was also directly related to tissue levels of Aroclor 1016 in the infant monkeys at birth. Thus, tissue dosimetry data is available to directly confirm the transplacental exposure of the new born monkeys to Aroclor 1016.

The reduced birth weight reported in Barsotti and van Miller (1984) was also used as the key study to obtain a RfD for Aroclor 1016 in the Drinking Water Criteria Document for PCBs (US EPA, 1989).

Neurobehavioral effects and transient dermal pigmentation attributed to Aroclor 1016 in the principal study have also been reported in other monkey studies with PCB exposure and in humans with PCB exposure. The RfD Summary provides a good overview of related,

supportive studies.

Selection of Uncertainty factors

The total UF of 100 appears appropriate based on the 4 areas of uncertainty considered in the RfD. A UF of 100, based on other considerations, was also applied in calculating the RfD for Aroclor 1016 in US EPA, 1989.

Weight of Evidence Conclusions

The RfD Summary supports the medium degree of confidence in the Study, Data Base, and RfD.

PART II Recommendations

At this time, based on my review and the above comments, I would favor Option A or B. Confirm the Aroclor 1016 RfD value as presented in the IRIS entry, but revise the text to include limitations, uncertainties, and other recommendations made during the peer review.

Stephen Safe

Stephen H. Safe

Review: Technical Review Workshop on the Reference Dose for Aroclor 1016

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Attachment 1 briefly outlines the principal and supporting studies which are being utilized to derive the RFD values for the PCB mixture, Aroclor 1016. Based on the known characteristics and composition of Aroclor 1016, there were some unusual and unexpected effects noted in these animals, namely:

(i)   the apparent dose-dependent decrease in body weight in control (528 g), low dose (486 g) and high dose (421 g) infants and

(ii)   hyperpigmentation in the low and high dose infants which did not persist.

There is good evidence that the effects noted above tend to be associated with aryl hydrocarbon (Ah) receptor-mediated responses caused by aromatic hydrocarbons including polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs). Moreover, examination of recent analytical data reported for Aroclor 1016 and the results of other feeding studies with Aroclor 1016 and other Aroclors indicate that the data reported by Barsotti and coworkers are problematic. A brief discussion of the inconsistencies between the studies utilized for deriving the RFD and other data is noted below. The major reason for this discussion is to point out the discordance between the observed data (*i.e.* hyperpigmentation and body weight loss) and the results expected for Aroclor 1016.

Aroclor 1016 - Analytical Data. Aroclor 1016 was prepared as a blend of PCBs which primarily contained di-tetrachlorobiphenyl congeners and virtually none of the higher chlorinated biphenyls which are found in mixtures such as Aroclor 1242 which has a similar chlorine content (by weight). This is clearly illustrated in the paper by Schulz et al. (*Environ. Sci. Technol.* 23, 852, 1989) who reported the high resolution analysis of Aroclors 1221, 1016, 1242, 1254 and 1260 and several Clophen mixtures. A similar chromatogram has been reported by Wolff and coworkers (*Toxicol. Appl. Pharmol.* 49, 199, 1982; *Environ. Health Persp.* 60, 133, 1985). Moreover, in the study by Wolff and coworkers, the gas chromatographic pattern observed in Aroclor 1016-exposed workers is similar to the pattern for Aroclor 1016. In contrast, the PCB gas chromatographic pattern observed for various fat extracts from Aroclor 1016-exposed monkeys (Barsotti and Van Miller)

shows at least 3 higher molecular weight PCBs which are not detected in Aroclor 1016. These peaks are routinely detected in fat samples from animals treated with higher chlorinated PCB mixtures suggesting that the animals used in this study were exposed to higher chlorinated PCBs (or PCB mixtures) in addition to Aroclor 1016. These PCBs may have been present as impurities in the feed or in Aroclor 1016 or in the monkeys used in this study. These analytical data suggest that the animals used in the Barsotti and Van Miller study were exposed to Aroclor 1016 and higher chlorinated PCBs and therefore standard setting (i.e. RFD) for Aroclor 1016 based on this study is problematic.

Aroclor 1016: Predicted versus Observed Toxicities. High resolution GC analysis of Aroclor 1016 (*Environ Sci. Technol.* 23, 852, 1989) also shows that relatively low levels of the "dioxin-like" (Ah receptor agonists) coplanar and monoortho coplanar PCBs are present in these mixtures compared to that observed for higher chlorinated commercial PCBs. Using a toxic equivalency factor (TEF) approach, "dioxin" or toxic equivalents (TEQs) for Aroclor 1016 are low (< 1 ppm?) compared to the values for Aroclors 1242 (696 ppm), 1254 (146 ppm) and 1260 (53 ppm) (Safe, *C.R.C. Crit. Rev. Toxicol.*, in press, 1994). For example, recent studies in my laboratory have determined the dose-response induction of hepatic microsomal EROD activity (an Ah receptor-mediated response) in female Sprague-Dawley rats. The results showed that at a dose of 50 mg/kg only minimal induction was observed for Aroclor 1016. The relative potencies of the Aroclors roughly paralleled their TEQs (Safe, 1994 and unpublished results).

EROD Induction in the Rat by Aroclors.

Treatment	dose (mg/kg)	EROD Activities (pmol/mg/min)			
		0	0.5	5.0	50
Aroclor 1260		14 ± 3	16 ± 7.3	34 ± 13	149 ± 78
Aroclor 1254		80 ± 16	93± 30	218 ± 177	4243 ± 524
Aroclor 1242		78 ± 11	104 ± 31	465 ± 545	2692 ± 1293
Aroclor 1016*		82 ± 6.6	84 ± 7.3	76 ± 16	124 ± 20

* induction is increased at higher doses and this may be related to induction of CYP1A2.

Not surprisingly, the results reported in I.A.4. ADDITIONAL STUDIES/ COMMENT (ORAL RFD) of Attachment 1 show that the typical "dioxin-like" effects of Aroclor 1016 such as body weight loss and hyperpigmentation are not observed whereas in studies with higher chlorinated PCBs, these effects

are reported. This point is noted by Barsotti and Van Miller (pp. 40 and 41); however, they do report some decreased body weights and hyperpigmentation suggesting that these effects may be associated with the higher chlorinated PCBs which have contaminated this study.

In my opinion, there is a problem in setting an RFD value for Aroclor 1016 when some of the observed responses may be associated with PCBs other than Aroclor 1016.

Richard Seegal

Part One: Reviewer Comments.

Charge #1. Selection and Use of Non-human Primate (NHP) Reports as the Primary Basis for Aroclor 1016 RfD.

The reports listed in Attachment #1 (Barsotti and van Miller, 1984; Levin *et al.*, 1988; Schantz *et al.*, 1989; Schantz *et al.*, 1991) provide in-depth analysis of the reproductive and neurobehavioral consequences of perinatal exposure of *Macaca mulatta* (rhesus macaque) to Aroclor 1016. Because the developing organism is most sensitive to exposure to putative toxicants (Tilson *et al.*, 1990) and because the NHP metabolizes polychlorinated biphenyls (PCBs) in a manner similar to humans (Matthews and Dedrick, 1984), these studies provide an appropriate basis for setting RfDs for Aroclor 1016.

Although these studies originated from a single laboratory and have not been replicated in other laboratories, the dose-related deficits in birth weight and discrimination reversal learning are sufficient to conclude that low-dose perinatal exposure to Aroclor 1016 may induce adverse health effects in humans. This conclusion is based on the following lines of evidence.

a. Perinatal exposure to 1 ppm of Aroclor 1016 resulted in significant decreases in birth weight (Barsotti, Thesis, Table 6-3, p. 209). Decreases in birth weight were reported only in the high-dose animals. However, examination of the summary data in that table suggests that there are also significant differences between the high and low dosed offspring (422 g vs. 491 g) -- i.e. a dose-response relationship. Thus, General Electric's (GE) comments that control animals were 'not adequately matched to experimental animals, had longer to acclimate to laboratory conditions and may have been obtained from different geographic sources' appear to be unfounded since birth weight differences exist between low and high dose 1016 animals obtained at the same time and maintained in the laboratory for the same time period.

b. Errors due to cross contamination of Aroclor 1016 chow with either polybrominated biphenyls (PBBs) or Aroclor 1248 were identified and appropriately handled. GE states that 'congeners with relative retention times (RRTs) of 125 and 146 were present in both the milk of monkey mothers and infants' adipose tissue'. Barsotti, in her thesis, noted that primate chow contained 1-50 ppb of Aroclor 1248. This level of contamination is not uncommon and most likely reflects low-level contamination of the primate chow. If this was the source, all NHPs, including controls, would have been exposed to the same contaminants. If the 1248 congeners were present in the original

Aroclor 1016 mixture, they were present at concentrations of between 1 and 50 **ppb**. At that level of contamination, the high dose Aroclor 1016 animals, exposed to 1 **ppm** of Aroclor 1016, would have been exposed, at most, to 50 parts per **quadrillion** of congeners derived from Aroclor 1248.

Inadvertent exposure of low dose Aroclor 1016 animals to PBBs was detected when adipose and milk samples were analyzed for PCBs. These animals were withdrawn from the study and any potential concerns over co-exposure are not relevant to addressing the 'fitness' of these studies in setting RfDs for Aroclor 1016.

c. Concerns over possible erroneous dosing of either or both Aroclor 1248 and Aroclor 1016 exposed animals may be due to differences in the ability of congeners present in these mixtures to induce hepatic enzymes and enhance metabolism of parent congeners.

GE states that 'newborn offspring from NHPs fed Aroclor 1248 in their diet had an average level of PCB in skin-adipose of 2.8 $\mu g/g$ while offspring from mothers exposed to 1 ppm of Aroclor 1016 had an average of 3.37 ppm'. GE concludes that this anomalous finding was due to erroneous dosing. However, there are two reasons for discounting these statements. First, the degree of variance in the measures of the PCB residues indicates that there may be no significant differences in body burdens. Secondly, concentrations of mono-*ortho* and coplanar congeners are much greater in Aroclor 1248 than in Aroclor 1016 (Hong *et al.*, 1993). Based on toxic equivalent factors (TEFs), (Safe, 1987), Aroclor 1248 contains approximately 300 times the TEF equivalents of Aroclor 1016 (Hong *et al.*, 1993). In addition, Aroclor 1248 exposed animals received 2.5 times the dose level of Aroclor 1016 animals, resulting in approximately a 750 fold greater exposure to PCB congeners that induce aryl hydrocarbon hydroxylase activity and enhance the metabolism of mono-*ortho* and coplanar PCBs. Furthermore, the three persistent congeners present in Aroclor 1016 (2,4,4'; 2,4,2',4' and 2,5,2',5') are also present at similar concentrations in Aroclor 1248 and would accumulate at the same rate. Based on these differences in metabolic potential between Aroclor 1248 and Aroclor 1016, it is highly unlikely that erroneous dosing, in either the Aroclor 1248, or more importantly, in the Aroclor 1016 exposed animals is responsible for the observed differences in skin/adipose concentrations of PCBs.

d. Adult exposure of Macaca nemestrina, pig-tailed macaques, to higher levels of Aroclor 1016 resulted in significant decreases in regional brain dopamine (DA) concentrations (Seegal *et al.*, 1991). Although these doses (0.8, 1.6 and 3.2 mg/kg/day) were significantly higher than those used in the Barsotti-derived animals, chemical analyses of serum concentrations of PCBs demonstrated that

these levels were similar to those seen in workers occupationally-exposed at the GE Fort Edwards factory (Lawton *et al.*, 1985). Furthermore, when similarly treated high-dose animals were removed from exposure for 24 weeks, brain and serum levels of PCBs decreased dramatically, in the absence of any return to control levels for brain DA. These findings are important since experimental alterations in brain DA concentrations have been shown to alter learning and memory process in both NHPs and rodents (Sawaguchi *et al.*, 1988; Archer *et al.*, 1988).

e. Chemical analysis of PCB residues in the brains of Aroclor 1016 sub-chronically treated adult NHPs reveal the presence of only three congeners (BZ #28, 47 and 52) (Seegal *et al.*, 1990). These findings are remarkably similar to the tissue residues seen in both adult and perinatally-exposed Aroclor 1016 animals by Barsotti (Thesis; Barsotti and van Miller, 1984). When cells in culture were exposed to these congeners, either alone or as a mixture that reflected the congener ratios seen in NHP brain, there were significant decreases in cellular DA concentrations (Seegal *et al.*, 1990). Further studies by Shain *et al.* (1991) have demonstrated that lightly chlorinated, *ortho*-substituted congeners, but not dioxin-like congeners, also significantly reduce cellular DA concentrations.

In addition, in studies underway, perinatal exposure of rats to 2,4,2',4' results in significant decreases in brain DA concentrations, reinforcing the finding that congeners derived from Aroclor 1016 are capable of altering central nervous system biogenic amine function. In turn, these neurochemical changes would be likely to affect the neurobehavioral dependent variables.

Charge #2. Selection of Critical Effects: Low Birth Weight versus Neurobehavioral Changes.

Perinatal exposure to 1 ppm of Aroclor 1016 resulted in a 15-20% decrease in body weight. However, there were greater absolute decreases in body weight between the low dose and high dose animals than between controls and low dose animals. These results suggest that the Aroclor 1016 induced decreases in body weight would have been evident even without comparisons to the control animals.

A more sensitive measure of perinatal exposure to Aroclor 1016 is provided by examination of the discrimination reversal data presented in papers by Schantz and Levin. In these papers the investigators determined that exposure to 1 ppm of Aroclor 1016 resulted in a **2.5 fold** increase in the number of original learning trials required for perinatally-exposed NHPs to reach a criterion of 9/10 correct trials. The magnitude of these changes are obviously much greater than those seen for alterations in birth weight and strongly suggests that alterations in behavior may be a more sensitive

measure of toxicant exposure than decreases in body weight. Indeed, NHPs with lesions in the dorsolateral area of the prefrontal cortex show a pattern of deficits that are very similar to those observed in the 1 ppm Aroclor 1016 exposed offspring (deficits on original learning and early reversals, but no deficits on later reversals). The similarity between the behavioral deficits induced by perinatal exposure to Aroclor 1016 and discrete lesions of the prefrontal cortex emphasize the validity of the discrimination reversal task in detecting alterations in cognitive function in NHPs and minimize concerns over the lack of statistically significant differences observed between the 1 ppm Aroclor 1016-exposed offspring and control animals on later reversal problems. This point needs to be further developed and stressed.

Charge #3. Uncertainty Factor Analyses for Aroclor 1016.

In Attachment #5 'Charge to Reviewers for the RfD for Aroclor 1016' four areas of uncertainty are assigned factors ranging from 1 to 10. The RfD work group has assigned uncertainty factors (UF) of three for all areas of uncertainty resulting in a total UF of less than 100. The total UF is less than the average for other agents examined by the RfD. However, it is suggested the UF_D and UF_S be further reduced because of the following evidence.

For UF_D a factor of three was assigned because studies 'relating to male reproductive effects and two-generation reproductive studies were not available'. All offspring were sired by unexposed males (Schantz *et al.*, 1991) and hence the possibility of male-induced reproductive and neurobehavioral deficits can be ruled out. Furthermore, statistical analyses of 'paternity effects' were conducted and no evidence of male-induced effects in offspring were found. Hence, there is no statistical evidence that possible male-induced reproductive alterations were responsible for any of the observed effects on birth weight or neurobehavioral change. I suggest that UF_D be changed from three to two. If two-generation reproductive studies had been carried out the UF_D could have conceivably resulted in further decreases to one.

For UF_S, an uncertainty of three was assigned because the duration of exposure was not of sufficient duration to warrant being called a 'true' chronic study. However, exposure to 1 ppm of Aroclor 1016 for 21.8 months appears to be of sufficient duration to result in steady state levels being reached in the mothers. This statement is based on the lack of statistically significant change in Aroclor 1016 milk concentrations in the 1 ppm exposed female NHP throughout the four month period of lactation. If steady state levels had not been reached, continued exposure to Aroclor 1016 during lactation would have resulted in a further elevation in Aroclor 1016 concentrations. Thus, the

statistically significant doubling of Aroclor 1016 milk concentrations seen in the 0.25 mg group argues that steady state levels had not been achieved in the lower dose animals.

Charge #4. Weight of Evidence Conclusions: Data Consistent with RfD Conclusions.

Conclusions based on review of Attachment #1 and the four key papers provide sufficient weight of evidence that perinatal exposure to Aroclor 1016 may yield significant adverse health effects in humans. This statement is based on the following lines of evidence

a. Perinatal exposure to 1 ppm Aroclor 1016 results in significant decreases in birth weight of exposed offspring and a 2.5 fold increase in the number of trials required to reach criterion on a discrimination reversal task. These data alone, should be sufficient to reach the conclusion that the RfD for Aroclor 1016 is scientifically valid.

b. Additional data gathered in adult NHPs exposed to Aroclor 1016 demonstrates that congeners present in this commercial mixture are sufficient to significantly alter brain DA concentrations and that these decreases persist following removal of the animals from exposure (Seegal et al., 1994). Experimental alterations of DA concentrations in the prefrontal cortex of NHPs resulted in deficits on cognitive tasks similar to those seen with exposure to Aroclor mixtures, reinforcing the importance of the findings that exposure of NHPs to Aroclor mixtures, including Aroclor 1016, induce cognitive deficits by altering brain concentrations of DA.

Data Less Consistent with RfD Conclusions.

Although 'there is difficulty in assessing human response-exposure to a mixture of congeners', data gathered by Seegal et al. (1991, 1994) following sub-chronic exposure of NHPs to Aroclor 1016, as well as the original chromatographic data from Barsotti (Thesis) ('peaks with RRTs of 37, 47 and 70 comprised the majority of the PCBs representing approximately 80% of the Aroclor 1016') demonstrate that there is a selective accumulation of a small number of Aroclor 1016 congeners. Indeed, Schantz et al. (1991) state that the RRT peak 37 consists 'primarily' of 2,4,4' while RRT peak 47 includes 2,4,2',4' and 2,5,2',5'. Using high-resolution glass capillary gas chromatography, we (Seegal et al., 1990) have shown that congeners accumulating in the NHP following adult exposure to Aroclor 1016 are 2,4,4'; 2,4,2',4' and 2,5,2',5'. Thus, the animals exposed by Barsotti et al to Aroclor 1016 accumulated the same congeners. Although in-vivo exposure to these three congeners has not yet been conducted, we have shown that in-vitro exposure to the three congeners that accumulated in NHP brain significantly reduced cellular DA concentrations. We suggest that chemical analyses of target

organs or body burdens following exposure to complex mixtures of congeners will provide more insight to the toxicological actions of the congeners and considerably less confusion than examination of congeners in the original dosing mixture.

Part Two: Recommendations.

I prefer a combination of Options A and C for the following reasons.

Based on statements made in the above text, I strongly feel that the criticisms raised by General Electric Corporation concerning the possible cross-contamination of the diets and the use of inappropriate control groups are not based on an accurate reading or interpretation of the data presented in the key articles. Thus, Option A would be the appropriate recommendation.

However, the statistically-significant deficits seen in the Aroclor 1016 exposed animals during the original learning of the discrimination reversal learning tasks strongly suggests that the neurobehavioral data should be incorporated or 'factored' into the RfD for Aroclor 1016. Hence, the reason for also recommending Option C.

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APPENDICES F-G

OBSERVERS AND OBSERVER MATERIALS

APPENDIX F

FINAL OBSERVER LIST

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APPENDIX G

OBSERVER COMMENTS

The Composition of the PCB Residues in Aroclor 1016/1248-Dosed Rhesus Monkeys as Indicated by Barsotti's Packed Column Gas Chromatograms

by John F. Brown, Jr.

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During the May 24, 1994 discussions of the U.S. EPA Technical Review Workshop on the Reference Dose (RfD) for Aroclor 1016, members of the Review Panel repeatedly raised questions regarding the interpretation and interpretability of the gas chromatograms (GCs) published by Barsotti and Van Miller in 1984. Since I have done a great deal of work in PCB GC data analysis and interpretation during the past decade, I should like to address some of the questions raised, and also indicate what sort of conclusions can be drawn from the available GC data.

1. Identification of PCB Congeners Giving Peaks Resolved on an SE-30 Packed Column. Many different types of PCB compositions have now been characterized by both low resolution packed column GC, which was in general use in the 1970's, and high resolution capillary GC, which has been increasingly used in recent years. Comparisons between the two types of GCs show that for the commercial PCB products (e.g., Aroclors), the observed packed column peaks (generally identified by relative retention times (RRTs), relative to DDE=100) usually represent envelopes of 2-6 individual PCB congener peaks, most of which are resolvable on suitable capillary columns. This means that it is generally not possible to assign a packed column Aroclor peak to an individual PCB congener. However, the PCB residues in higher animals that have been dosed with the lower Aroclors, such as 1016, 1242, 1248, or even 1254, have a greatly simplified congeneric composition, owing to the metabolism and elimination of most of the lower PCB congeners originally present. As a result, most of the packed column GC peaks exhibited by animal tissue residues are given by single congeners, and the remainder are mostly simple binary mixtures. The identities of the metabolically-resistant congeners giving the more commonly observed SE-30 peaks from some or all of these Aroclors have been reported (Brown et al., 1989) as follows:

SE-30 RRT	Major Component (No.)	Minor Component (No.)
37	2,4,4' CB (28)	---
47	2,2',4,4' CB (47)	2,2',5,5' CB (52)[a]
70, 70A[b]	2,4,4',5 CB (74)	2,3',4,4' CB (66)[b]
78	2,3,4,4' CB (60)	---
84	2,2',4,4',5 CB (99)	2,2',4,5,5' CB (101)[a]
98	2,2',3,4,4' CB (85) + DDE	
125	2,3',4,4',5 CB (118)	
146	2,3,3',4,4' CB (105)	2,2',4,4',5,5' CB (153)[c]
174	2,2',3,4,4',5' CB (138)	2,3,3',4',5,6 CB (163)

a. Observed only in lightly metabolized specimens.
b. PCB No. 66 often resolved from PCB No. 74 even on packed columns.
c. Resolved from PCB No. 105 on mixed phase (pesticide) packed columns, although not on SE-30; generally a dominant rather than a minor, component of Peak 146 in environmental samples.

Generally speaking, the quantitation of the packed column peaks, e.g., by the products of the peak area and peak response factor, as in the method of Webb and McCall (1973), is not much less precise than that for capillary GC peaks. As a result, even though Barsotti's analyses were done by a now-obsolete procedure, there is no methodological basis for challenging either the magnitudes of the various PCB peaks observed or the identities of their major components. The Webb and McCall procedure used should have required the determination of the amount of PCB associated with each individual packed column peak, and then adding these numbers to determine the total PCB present. If the original records still exist, they should contain listings for the levels of each individual peak.

2. Characterization of Operative Metabolic System(s) from Residual PCB Congener Distribution. It will be noted from the above table that the residual PCB congeners in a well-metabolized specimen consist almost entirely of congeners with 4,4'-substitution. Within this group, however, important variations in relative persistence can occur. This was first reported by Masuda et al. (1974), who noted that in chloracne patients who had ingested rice oil (yusho) contaminated with PCB-PCDF mixtures, packed column Peaks 37, 70, 78, and 125 were either missing or sharply reduced, and Peak 146 somewhat reduced as well, whereas Peaks 84, 98+DDE, and 174 were unaffected, giving a peculiar GC pattern termed "Pattern A." This was later recognized to have resulted from the increased metabolism of the mono-*ortho* PCB congeners (e.g., PCB Nos. 74, 60, 118, and 105) by the PCDF-induced cytochrome P4501A isozymes operating in addition to the cytochromes with P4502B-like selection patterns which are responsible for most of the PCB metabolism seen in normal humans, mice, eels, crabs, etc. (Brown et al., 1989; 1992). Neither humans who were occupationally exposed to Aroclors 1016, 1242, and 1254 (Brown et al., 1989; 1991) nor mice dosed with Aroclor 1254 (Anderson et al., 1991) have shown "Pattern A" tissue residues; however, in rats dosed with Aroclors 1242 or 1254, which are known to be good inducers of P4501A isozymes in that animal, Pattern A is strongly developed (Brown et al., 1994). Since Aroclor 1248 is known to induce P4501A activity in the closely related cynomolgus monkey (Iverson et al., 1982) one would also expect to find Pattern A in the monkey PCBs as well. However, a recent examination of the residues in rhesus monkeys that had been chronically dosed with Aroclor 1254 (Mes et al., 1989; Arnold et al., 1990; Brown, Arnold, Mes, and Bryce, 1994, manuscript in preparation) showed exactly the opposite pattern, i.e., a preferential loss of di-*ortho*-substituted congeners (and in an unusual selection pattern at that) relative to the mono-*ortho* congeners. This unusual metabolic process was apparently able to overcome any tendency for preferential mono-*ortho* congener removal by P4501A, and leave tissue residues dominated by mono-*ortho* 4,4'-substituted congeners, e.g., PCB Nos. 105, 118, and 156 from Aroclor 1254. In the Barsotti monkeys, it may be noted that four of the five major peaks, i.e., Peaks 37, 70, 125, and 146 are given by mono-*ortho*'s. Peak 47 is not, but it is also the peak that dwindles away after the cessation of dosing (Barsotti and Van Miller, Figure 4). Thus, the particular set of 4,4'-substituted PCB congeners that remain in the Barsotti monkeys are those that would be expected in view of those observed by both packed column and capillary GC in 1254-dosed monkeys.

3.   Calculation of Relative Contributions of Aroclor 1016 and 1248 to the PCB Residues in Barsotti's Monkeys. During the panel discussions on May 24, Professor Hartung correctly pointed out that to determine the relative proportions of Aroclors 1016 and 1248 administered to the Barsotti's monkeys from the composition of the residual PCBs in the monkey tissue would require quite elaborate pharmacodynamic calculations. I would also add that the clearance rate constant data needed to perform such calculations, although now known for the human, are not known for the monkey.

However, for purposes of estimating toxic effects, it is probably more important to determine the relative contributions to retained body burden, rather than administered dose, since it is the retained PCBs that determine the internal exposure of the animal.

Figure 1 shows a capillary GC for the brain PCB residues remaining in a pig-tailed macaque that had been dosed with Aroclor 1016, as reported by Seegal et al. (1990). (I have been informed by Seegal's co-author, Dr. Brian Bush, that the chromatograms for other tissues from these animals were virtually identical to the one shown as Figure 1.) It will be noted that the Seegal monkey's chromatogram shows individual congener peaks for 2,4,4' CB, corresponding to packed column Peak 37; for 2,2',5,',5'- and 2,4,2',4 CB, corresponding to the marginally bifurcated packed column Peak 47; and some very small unlabelled peaks in the region expected for packed column Peak 70. The relative sizes of the peaks corresponding to packed column Peaks 37, 47, and 70 are approximately as they are in the Aroclor 1016 standard (Barsotti and Van Miller, Figure 1). By contrast, the packed column GCs of the PCBs in Barsotti monkeys (Figures 2, 3) show additional strong peaks with RRTs 70, 125, and 146, and weaker ones at RRT 174 and in the RRT range 78-100. The additional peaks seen are those of the 4,4'-substituted tetra-, penta- and hexachlorobiphenyls present in Aroclor 1248 or 1254 (see above table) and in approximately the relative proportions expected from the packed column chromatograms of Aroclor 1248 (Webb and McCall, 1973). This Aroclor 1248 reference GC also shows, however, that any contribution of Aroclor 1248 to the total would have to include substantial contributions to Peaks 37 and 47 as well as giving Peaks 78-98, 125, 146, 174, and most of Peak 70.

In order to estimate the relative contributions of Aroclors 1016 and 1248 to the Barsotti monkey GCs, I first focused on GCs (e.g., Barsotti Figure 4) where all of the peaks appeared to be on scale, so that they could be seen in their entirety. I then estimated the relative area of each peak in mm^2 by multiplying its height by its half-width. Lacking any data on individual peak response factors for the GC instrument used, I assumed that the quantity of PCB in each peak was proportional to peak area. Any resultant errors were, however, at least partly cancelled by applying the same procedure to the peaks in the Aroclor standards. I then calculated, by the method of successive approximations, the relative contributions of Aroclor 1016 and 1248 to the observed GC, assuming that each of the persistent peaks would appear in the GC in the same proportions as in the original Aroclor. The results for the relatively highly metabolized (low Peak 47) PCBs of Barsotti and Van miller's infant monkey 4 mo. after weaning (their Figure 4) are given below:

Peak No.	Total Area (mm^2)	Attrib. to Aro. 1016	Attrib. to Aro. 1248
37	102	55	47
47	6	2	4
70	106	8	98
78-98	18·	---	18
125	49	---	49
146	29	---	29
174	8	---	8
Total (%)	318 (100%)	65 (20%)	253 (80%)

The results indicated that about 80% of the tissue residues in the infant monkey remaining after 4 mo. recovery came from Aroclor 1248 rather than 1016. A similar calculation for the same infant monkey at the time of weaning from Figure 3 was a bit more difficult because Peaks 37 and 70 were off-scale; however, guesses as to their areas from their widths suggested an Aroclor 1248 contribution around 60%. One might be concerned, however, over the apparently large size of Peak 70 in Figure 3, which suggests the possibility that it might be coming from a non-PCB contaminant. If, as an extreme case, one were to assume that Peak 70 came entirely from a contaminant, Peaks 37 and 47 entirely from Aroclor 1016, and Peaks 78-174 from metabolized Aroclor 1248 residues, the contribution of such residues to total peak area in Figure 4 would be 49%, and to those of Figure 3, 35%. In short, the range of estimated contributions of Aroclor 1248 to the observed monkey PCB GCs was 35-80%, with the smaller numbers requiring an assumption of analytical error concerning the true levels of Peak 70.

Lest anyone miss the point that tissue residues in at least some of the 1016-dosed monkeys must have come largely from Aroclor 1248, I attach a copy of an old SE-30 packed column chromatogram of Aroclor 1248, as shown as Figure 6 in the original Webb and McCall paper of 1973, which was cited by Barsotti and Van Miller as the basis for their analytical procedure. This chromatogram shows the metabolically-resistant Aroclor 1248 peaks at RRT 37, 47, 70, 125, and 146 in just about the same relative proportions as in the monkey of Barsotti's Figure 3, whereas Seegal's monkey (Figure 1), which received only Aroclor 1016, showed significant levels of only the congeners corresponding to packed column peaks 37 and 47. In short, Barsotti and Van Miller's published chromatograms are simply incompatible with their statement that the PCB given to the animals was essentially Aroclor 1016.

4. Sources of the Higher PCBs. The source(s) of the Barsotti monkeys' PCBs of Peaks 70-174 remain problematic. One identified contributor is the monkey chow, which did indeed contain low levels of PCBs. However, the adipose PCB level in control monkey No. 33 was only 0.36 ppm, and the GC pattern (Barsotti and Van Miller Figure 6) showed little Peak 70 and Peak 146 > Peak 125, which was more suggestive of the metabolized residues of an Aroclor 1254-like PCB composition than Aroclor 1248. Thus, neither the level nor pattern of the higher PCB peaks (i.e., those in the RRT range 70-174) in the control monkey that got the same chow as the test animals seem consistent with the hypothesis that the chow was the major sources of the higher PCBs seen in the test animals. Instead, we must conclude that either Aroclor 1248 was inadvertently mixed into the feed, along with the Aroclor 1016 that was on test, or else that the monkeys in the 1016 test groups were inadvertently given some of the Aroclor 1248-dosed feed that

was being given to other test groups at the site. In view of the facts that one of the original three Aroclor 1016 test groups was found, according to Barsotti's thesis, to have received substantial quantities of PBB, which was also on test at the same time, the latter possibility requires very serious consideration, and suggests that the U.S. EPA should make an examination of the animal feeding records, as well as getting analyses on any residual samples of the feed used. The alternative possibilities, namely, that the higher PCBs came from either the chow as purchased or from an error in the selection of the Aroclor added to it in preparing the feed, could be easily assessed by checking out the relative levels of Peaks 125 plus 146 in the monkeys of the various study groups.

If it becomes necessary to obtain new chromatograms of stored monkey tissues in order to make this assessment, or to assess the possibility that much of the Peak 70 arose from a non-PCB impurity that was not removed by the analytical clean-up procedure used, General Electric would be willing to provide or support the appropriate GC analyses, subject to U.S. EPA involvement and overview.

5. Toxicological Implications. The hyperpigmentation and neurobehavioral effects observed in Barsotti's Aroclor 1016/1248-dosed monkeys were more conspicuously seen in the 1248-dosed monkeys, and have been already attributed to the presence of toxic congeners such as 2,4,5,3',4'-pentachlorobiphenyl (Levin, Schantz, Bowman, 1988). Since these, and other, congeners with dioxin-like activities are lacking in Aroclor 1016, we must conclude that the hyper-pigmentation and neurobehavioral effects arose from the inadvertent presence of Aroclor 1248, rather than from the Aroclor 1016 itself.

Whether the weakly dioxin-like activity of the higher PCBs present in the 1016-dosed monkeys could also explain the seemingly reduced birth weight is still problematical. In the Canadian studies of 1254-dosed rhesus monkeys there were extensive observations of dioxin-like effects on the fingernails, meibomian glands, and reproductive success, but reportedly no birth weight depression. However, in view of the uncertain comparability between the test groups and controls, the reality of the association between Aroclor 1016 and birth weight depression remains uncertain.

In summary, advances in the understanding of PCB metabolism and chromatography that have occurred in recent years have significantly increased the interpretability of the sort of gas chromatograms shown in the Barsotti and Van Miller report of 1984. It is now apparent that the internal PCB exposures experienced by the monkeys that were given Aroclor 1016 were roughly half derived from higher Aroclors rather than 1016 itself, and that the unambiguous toxic effects seen were characteristic of those higher Aroclors. Accordingly, it seems most inappropriate to be using any results observed in this seriously flawed study as a basis for an official agency position on the risks posed by Aroclor 1016.

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Atts.

Peak 37

(2,4,4'

Peak 47

2,5,2',5'

2,4,2',4'

Peak 70 ?

15

90

TIME (min)

or adipose
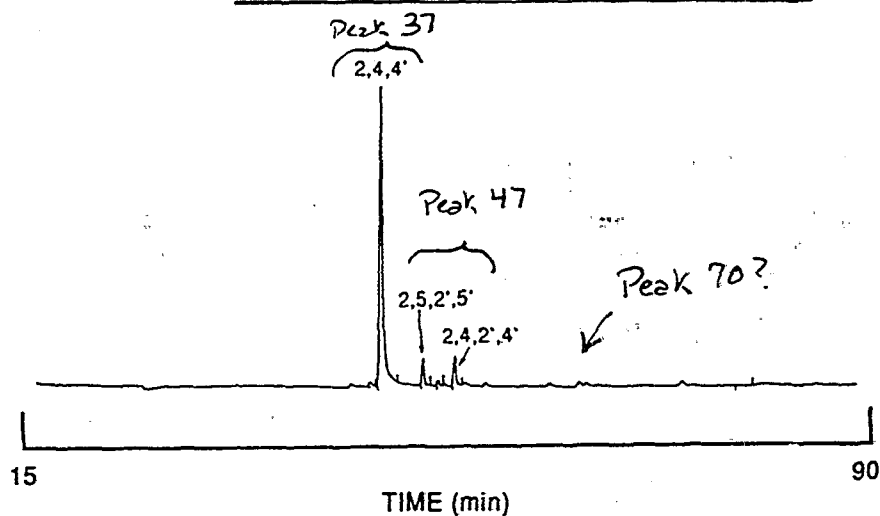
FIG. 1. Glass capillary gas chromatogram of Aroclor 1016 congeners present in the caudate of the nonhuman primate, *Macaca nemestrina*, following exposure to 3.2 mg/(kg·day) Aroclor 1016 for 20 weeks.
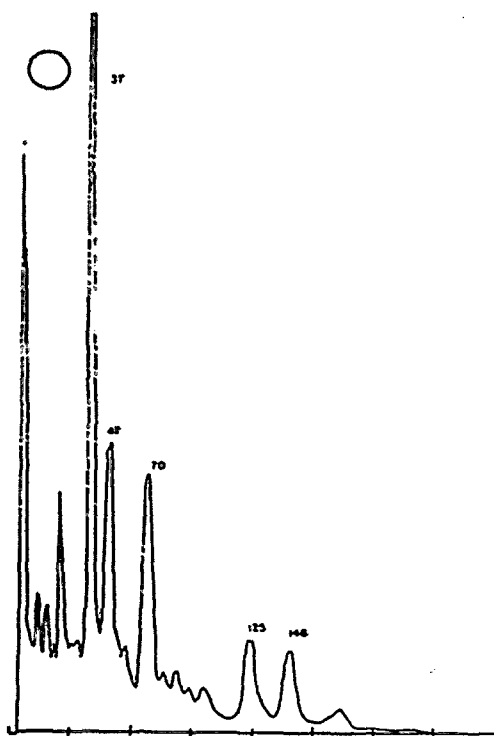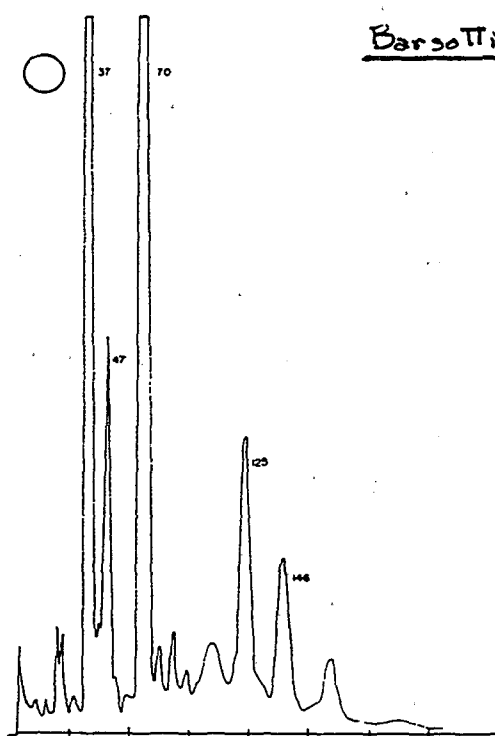
Barsotti and Van Miller, 1984

FIG. 2.

FIG. 3.

Fig. 2. Gas chromatographic tracing of a subcutaneous fat sample from monkey No. 79 after receiving 1.0 ppm Aroclor 1016 in the diet for 18 weeks. The level of Aroclor 1016 on a whole tissue basis was 2.27 ppm and 3.25 ppm on a lipid basis. (Attenuation = 8; Temperature = 200°C).

Fig. 3. Gas chromatographic tracing of a mesenteric fat sample taken from monkey No. 79's infant AG81 (1.0 ppm Aroclor 1016 group) at the time of weaning. The level of Aroclor 1016 on a whole tissue basis was 10.43 ppm and 31.31 ppm on a lipid basis. (Attenuation = 32; Temperature = 200°C).
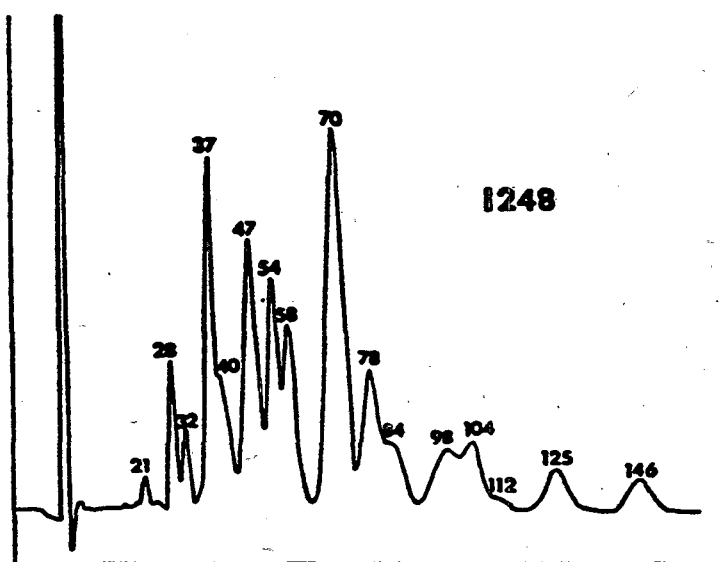
Figure 6. EC chromatogram of Aroclor 1248 chromatographed
on SE-30 with a Ni-63 detector operated in the DC mode.
The peak identification numbers correspond to the retention
time relative to p,p'-DDE=100.

From Webb and McCall, 1973.