IEHR

Institute for Evaluating Health Risks

President John A. Moore

Suite 608
1101 Vermont Avenue, NW
Washington, DC 20005
Tel: (202) 289-8721
Fax: (202) 289-8530

NAS-Beckman Center
Irvine, California

REASSESSMENT OF LIVER FINDINGS in FIVE PCB STUDIES IN RATS

July 1, 1991

REASSESSMENT OF LIVER FINDINGS IN PCB STUDIES IN RATS

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Pathology of Chronic Polychlorinated Biphenyl (PCB) Feeding in Rats. Schaeffer, E., Greim, H., & Goessner, W. Tox and Applied Pharm. (1984) 75:, 272-288.

It is not proper to continue a policy which does not consider data, developed subsequent to the initial judgement, that demonstrates other formulations are either not carcinogens or at best, weak carcinogens. There is precedent for such action; several years ago the Science Advisory Panel, which advises the State of California on cancer designations under Proposition 65, voted to recommend Aroclor 1260 as a carcinogen rather than all PCBs.

Utilize all available data when calculating cancer potency for PCB mixtures that have 60% chlorination

When one compares the consensus pathology diagnoses across four studies, in three different laboratories, there appears to be no scientific basis for continuing the practice of selecting only part of the available data for deriving potency estimates. Using this approach, the current EPA value of 7.7 would be replaced with a value of approximately 1.9.

I am not asking you to focus on an issue that is only of arcane scientific interest. The current cancer policy is clearly overstating the cancer risks associated with many exposures to PCBs in the environment. In a number of instances it is driving regulatory decisions that, by any standard are a major economic impact for, at best, trivial public health gain. As an illustration, mixtures with 60% or greater chlorination comprised about 12% of total PCB sales in this country; yet current policy calculates all PCB exposure as if it were equivalent to Aroclor 1260. While PCBs in the environment undergo changes in composition none develops into the chemical "fingerprint" that identifies Aroclor 1260. Therefore, 88% of the PCB that was used is being treated as if it were a potent carcinogen when the data indicate that these lower chlorinated mixtures are either of markedly diminished potency or not carcinogenic at all!

A request to develop a risk assessment utilizing all pertinent data, I believe, is consistent with the Agency's stated goals of focusing on risks which represent true public health or environmental concern and of reducing the uncertainties in risk assessment by applying sound scientific knowledge.

I would be pleased to work with the Agency in explaining the results of this project and discussing alternative approaches to estimating PCB risks. A copy of the pathology reassessment and a letter sent to Agency colleagues which provides greater details on risk related issues is enclosed.

Sincerely,

/s/

John A. Moore

IEHR

Institute for Evaluating Health Risks

President John A. Moore

Suite 608
1101 Vermont Avenue, NW
Washington, DC 20005
Tel: (202) 289-8721
Fax: (202) 289-8530

NAS-Beckman Center
Irvine, California

July 1, 1991

The Honorable Erich Bretthauer
Assistant Administrator
Office of Research and Development
Environmental Protection Agency
401 M Street SW
Washington, DC 20460

Dear Erich:

The Institute for Evaluating Health Risks has just completed a project in which the pathological diagnoses in five key rat PCB studies were reassessed.[1] Based on the results of this reassessment, a copy of which is enclosed, these studies could then be analyzed for consistency of result and it could be determined whether the differences in tumor incidence and type were due to the differing levels of chlorination in the tested PCB mixtures. The analysis clearly indicates that a reconsideration of the Agency's traditional cancer risk policy is warranted.

In the studies that were reviewed in this project rats were chronically exposed to commercial PCB formulations with three different levels of chlorination. The results of the pathology reassessment are briefly summarized as follows:

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[1] The project, which was funded by General Electric, was managed by the Institute for Evaluating Health Risks; coordination of the pathology reassessment was performed by Experimental Pathology Laboratories Inc.

These specific studies were selected because they were utilized or discussed in previous EPA risk assessments and they represent the best studies for evaluating the cancer potential of these mixtures of chemicals.

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Institute for Evaluating Health Risks

President John A. Moore

Suite 608
1101 Vermont Avenue, NW
Washington, DC 20005
Tel: (202) 289-8721
Fax: (202) 289-8530

NAS-Beckman Center
Irvine, California

July 1, 1991

The Honorable Hank Habicht
Deputy Administrator
Environmental Protection Agency
401 M Street S.W.
Washington, DC 20460

Dear Hank:

The Institute for Evaluating Health Risks has just completed a project, funded by General Electric, in which the pathological diagnoses in five key rat PCB studies were reassessed. This reassessment is the consensus diagnoses of eminent pathologists who are particularly experienced in rodent liver tumors. Using these diagnoses the studies were then analyzed for consistency of result and to determine whether the differences in tumor incidence and type were due to the differing levels of chlorination in the tested PCB mixtures.

The analysis clearly indicates that a reconsideration of the Agency's traditional PCB cancer risk policy is warranted. The policy positions to which I refer are: 1) an assumption that all PCB formulations are probable human carcinogens; 2) the assumption that all PCB formulations have the same quantitative potency to cause cancer.

Both of these positions were initially established years ago when our knowledge base from which to determine the cancer potential of PCBs was meager. They represent the use of conservative default assumptions. However, since then new data and knowledge have accrued that have not been effectively incorporated into the PCB risk assessment. Data, when available, should have priority over default assumptions.

A revised Agency PCB cancer risk assessment should reflect the following:

<u>Develop separate risk assessments for each of the major PCB formulations.</u>

The reassessed data underscore that there are major differences in carcinogenic potential based on the degree of chlorination of the PCB mixture. While the results from studies of mixtures with 60% chlorination consistently report a high incidence of liver tumors, studies in rats which were fed mixtures with 54% or 42% chlorination did not detect statistically significant elevations of liver tumors.

reaffirmed that chronic dietary exposure of rats, in three different studies, to 60% chlorinated PCB formulations resulted in the development of benign and malignant liver tumors;

reaffirmed that chronic exposure of rats to a PCB formulation that was 54% chlorinated did not yield a statistically significant increase of either benign or malignant tumors;

revealed that rats chronically exposed to a PCB formulation that was 42% chlorinated did not develop any increase in malignant tumors or a statistically significant increase in benign tumors.

These reassessment results indicate that the following two traditional EPA PCB policy positions be reconsidered: 1) an assumption that all PCB formulations are probable human carcinogens; 2) the assumption that all PCB formulations have the same quantitative potency to cause cancer.

Both of these positions were initially established years ago when our knowledge base from which to determine the cancer potential of PCBs was meager. They represent the use of conservative default assumptions. However, since then new data and knowledge have accrued that have not been effectively incorporated into the PCB risk assessment.[2]

I believe that a revised PCB cancer risk assessment should reflect the following:

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[2] Because of insufficient data default assumptions commonly are a necessary component of a risk assessment. However, there is another policy position which should guide the decision that determines the use of defaults; that overarching policy should establish a clear bias for the use of data whenever it is available. In other words the operant policy position is to use data, the burden should lie on the risk assessor to clearly establish why available data should not be used before it can be replaced by a default assumption.

Develop separate risk assessments for each of the major PCB formulations.

The reassessed data underscore that there are major differences in carcinogenic potential based on the degree of chlorination of the PCB mixture. While the results from studies of mixtures with 60% chlorination consistently report a high incidence of liver tumors studies in rats which were fed mixtures with 54% or 42% chlorination did not detect statistically significant elevations of liver tumors. It is not proper to continue a policy which does not consider data, developed subsequent to the initial judgement, that demonstrates other formulations are either not carcinogens or at best, weak carcinogens. There is precedent for such action; several years ago the Science Advisory Panel, which advises the State of California on cancer designations under Proposition 65, voted to recommend Aroclor 1260 as a carcinogen rather that list all PCBs.

The tissue diagnoses of the expert group of pathologists should be used for risk assessment.

There are three factors that support the use of these consensus diagnoses:

1) it reflects the use of current pathology conventions that are endorsed by the National Toxicology Program and the Environmental Protection Agency;

2) it represents the consensus opinion of pathologists that are experienced in the evaluation of rodent bioassays; specifically liver tumors.

3) the results of the present review permits greater confidence that observed differences in tumor incidence and type in each study are due to differences in the test substances.

Utilize all available data when calculating cancer potency for PCB mixtures that have 60% chlorination.

There is no logical basis to continue the current practice of only using the results obtained in female Sprague Dawley rats. A comparison of the results of each of these studies[3] shows a striking similarity in the nature of the tumor response. It should be noted that three separate strains of rats were used and that the similarity of response is apparent when one compares female Sherman rats, male Wistar rats, and female Sprague Dawley rats. Male Sprague Dawley rats, while developing the same type of liver tumors, did so at a lower incidence. To assume that this reduced response reflects a generic tendency of male rats not to develop tumors is not supported by the data. The greatest incidence of liver tumors (91.2%) was observed in male Wistar rats. The results in male Wistar rats also do not support continuing the practice of censoring the male Sprague Dawley results from the calculation of a cancer slope factor.

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[3] Induction of Liver Tumors in Sherman Strain Rats By Polychlorinated Biphenyl Aroclor 1260. Kimbrough, R.D. et al, JNCI (1975) 55:6, 1453-1459.

Polychlorinated Biphenyl Induction of Hepatocellular Carcinoma in the Sprague Dawley Rat. Norback, D.H. & Weltman, R.H., Env Hlth Perspect (1985) 60:, 97-105.

Pathology of Chronic Polychlorinated Biphenyl (PCB) Feeding in Rats. Schaeffer, E., Greim, H., & Goessner, W., Tox & Applied Pharm. (1984) 75:, 272-288.

When using the results from each of these studies one should apply a consistent decision rule to the censoring of animals from studies; each author used a different convention in their publications. Observing the convention employed by the National Toxicology Program may be more appropriate and consistent for all studies.[4] The group size in several of these studies would increase if this recommendation were adopted.

Employing the geometric mean of the cancer potency factors of the four study groups, female Sherman, male Wistar, male Sprague Dawley, and female Sprague Dawley rats would reflect a less arbitrary use of all existing data. There is ample precedent for this approach in a number of Agency decisions. The geometric mean, using the re-evaluation results, would yield a cancer potency factor of approximately 1.9. The current value calculated by EPA is 7.7 using only the female Sprague Dawley rat.

The reassessment of the NCI study[5] clarifies the significance of "nodular hyperplasia"

This study which evaluated a PCB mixture with 54% chlorination, essentially reaffirmed the original findings that the bioassay did not show a carcinogenic response in either male or female F344/N rats. The group size at each treatment level was 24 rats.

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[4] Censor all rats that died during the first year of the study or censor rats that died prior to the diagnosis of the first tumor in a target organ; whichever date is earlier.

[5] Bioassay of Aroclor 1254 for Possible Carcinogenicity. NCI Carcinogenisis Technical Report Series, Number 38, 1978.

Utilizing the current pathology nomenclature the consensus diagnoses by the expert panel classified "nodular hyperplasia" lesions, a designation used in the original report, as nonneoplastic. Therefore, continuing to incorporate the incidence of nodular hyperplasia in a cancer potency calculation, as was done in the most recent Water Quality Criteria Document[6] would fail to have a supportable scientific basis.

Rather than exclusively focus on how to estimate a cancer potency factor for the 54% chlorination PCB mixture I would urge consideration of a more fundamental question; namely, the estimation of cancer potency from any negative study.

The reassessment of the pathology diagnoses of lesions in the liver of rats fed a PCB mixture containing 42% chlorination reveals that there is no statistically significant increase in tumors.[7]

This study, which was performed in parallel with a PCB mixture with 60% chlorination, has not been accorded the attention that it deserves from a risk assessment perspective.[8]

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[6] Drinking Water Criteria Document for Polychlorinated Biphenyls, April 1988, (PB89-192256) pp VIII-32 to VIII-35.

[7] Liver tumor incidence in controls 8/120 (hepatocellular adenoma 6/120, hepatocellular carcinoma 2/120). Liver tumor incidence in treated group 16/128 (hepatocellular adenoma 14/128, hepatocellular carcinoma 2/128). Fisher exact test, one tailed, p =.098). It is arguable that a two tailed test should be used given that a decrease in pituitary tumors and endocrine tumors was reported in several of these studies. A two tailed test would further erode the p value.

[8] Pathology of Chronic Polychlorinated Biphenyl Feeding in Rats. Schaeffer, E., Greim, H., & Goessner, W., Tox. & Applied Pharm. (1984) 75:, 272-288

Factors which underscore the value of this study include:

1) it is the only major study of a PCB mixture with this level of chlorination.

2) it has far better statistical power to detect an effect than do most bioassays, e.g., the number of animals studied were about two and a half times greater than required by EPA or used by the National Toxicology Program.

3) the selection of male rats as the test subject would not appear to be a limitation. A parallel group of male rats, fed a PCB mixture containing 60% chlorine, yielded a liver tumor incidence of 91%, the highest incidence reported in any of the studies that were reassessed.

4) the study duration was approximately 118 weeks, this is three months longer than the protocol requirements of either EPA or the National Toxicology Program. It is generally held that studies of longer duration favor the detection of tumors, particularly with these types of chemicals.

I am not asking you to focus on an issue that is only of arcane scientific interest. The current cancer policy is clearly overstating the cancer risks associated with many exposures to PCBs in the environment. In a number of instances it is driving regulatory decisions that, by any standard are a major economic impact for, at best, trivial public health gain. As an illustration, mixtures with 60% or greater chlorination were about 12% of total PCB sales in this country; current policy calculates <u>all</u> PCB exposure as if it were equivalent to Aroclor 1260.

While PCBs in the environment undergo changes in composition they do not develop into the chemical "fingerprint" that identifies Aroclor 1260. Therefore, 88% of the PCB that was used is being treated as if it were a potent carcinogen when the data indicate that these lower chlorinated mixtures are either of markedly diminished potency or not carcinogenic at all!

A request to develop a risk assessment utilizing all pertinent data, I believe, is consistent with the Agency's stated goals of focusing on risks which represent true public health or environmental concern and of reducing the uncertainties in risk assessment by applying sound scientific knowledge.

I would be pleased to work with the Agency in explaining the results of this project and discussing alternative approaches to estimating PCB risks.

Sincerely,

/s/

John A. Moore

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

REASSESSMENT OF LIVER FINDINGS IN PCB STUDIES IN RATS

PATHOLOGY WORKING GROUP REVIEW

Submitted to:

Institute for Evaluating Health Risks
Washington, DC 20005

Submitted by:

Experimental Pathology Laboratories, Inc.
Research Triangle Park, NC 27709

June 27, 1991

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

PWG PARTICIPANTS:

Dr. Jerry F. Hardisty
Experimental Pathology
Laboratories, Inc. (Chairperson)

Dr. W. Ray Brown
Research Pathology Services, Inc.

Dr. Ernest E. McConnell
Consultant

Dr. James A. Popp
Chemical Industry Institute
of Toxicology

Dr. Robert A. Squire
Johns Hopkins University

Dr. Jerrold M. Ward
Consultant

Dr. Deborah A. Banas
Experimental Pathology
Laboratories, Inc.
(Reviewing Pathologist)

REASSESSMENT OF LIVER FINDINGS IN PCB STUDIES IN RATS

PATHOLOGY WORKING GROUP REVIEW

NARRATIVE SUMMARY

INTRODUCTION

Polychlorinated biphenyls (PCBs) are compounds whose physical and chemical properties led to their widespread use for a number of commercial applications. Because of their extensive use in the past and their resistance to chemical and biological breakdown, PCBs are now widely distributed in the environment.

PCBs were manufactured commercially by the chlorination of biphenyl. The number and placement of chlorine atoms introduced into the biphenyl molecule determines each PCBs' structure. There are 209 possible PCB congeners or homologs. Commercial PCB formulations are composed of complex mixtures of individual PCBs rather than a single congener. The percent chlorine by weight increases as the average number of chlorine atoms per biphenyl is increased. The chlorine content of various commercial formulations differs according to the desired physical characteristics for specific applications (Siberhorn, et al., 1990).

A number of studies have been undertaken to assess the potential toxicity and carcinogenicity of commercial PCB preparations. The main target organ of PCBs is the liver. A number of investigators have studied the potential carcinogenic effect of various PCBs in the

liver of rats and mice. These studies have indicated that benign or malignant hepatocellular tumors and various nonneoplastic hepatic changes are observed in the liver of rodents when given at appropriate doses for extended periods of time. These studies also indicated that PCB mixtures with a high chlorine content are more potent in inducing neoplastic nodules and hepatocellular carcinomas than mixtures with less chlorination. In most of these studies, the criteria for diagnosis and nomenclature designations according to Squire and Levitt were used by the investigators to classify the hepatocellular changes (Siberhorn, et al., 1990).

In the recent past, there have been changes in the criteria and nomenclature for hepatoproliferative lesions of rats (Maronpot, et al., 1986). These changes resulted from the increased accumulation of data, as well as a better understanding of the mechanisms of toxicity and carcinogenesis. In light of these changes, it was considered reasonable to reexamine the livers from several earlier PCB studies to assess the risk posed by these compounds based on the current understanding of hepatic changes. At the request of the Institute for Evaluating Health Risks (IEHR), Experimental Pathology Laboratories, Inc. (EPL) assembled all liver slides from five (5) key chronic PCB studies for the purpose of reassessment of the liver findings following current diagnostic criteria and nomenclature. These studies included the following: Aroclor 1260 in female Sherman rats (Kimbrough, et al.,

1975); Aroclor 1260 in male and female Sprague-Dawley rats (Norback and Weltman, 1985); National Cancer Institute (NCI) Aroclor 1254 study in male and female Fischer 344 rats (NCI, 1977); Clophen A 60 in male Wistar rats (Schaeffer, et al., 1984); and Clophen A 30 in male Wistar rats (Schaeffer, et al., 1984). Additionally, the liver slides from male and female rats used in a reproduction study in male and female Sherman rats with Aroclor 1260, in which the exposure was limited to nine months, were reexamined to characterize the degree and extent of hepatic lesions resulting from subchronic exposure to Aroclor 1260 (Linder, et al., 1974). References for each of these studies are included in Appendix F. The concurrent review of these studies provide a unique opportunity to compare the incidence, type and severity of hepatic lesions observed in each.

A summary of the experimental design for each of the studies included in this current review is presented on the following table.

SUMMARY OF EXPERIMENTAL DESIGN
PCB STUDIES IN RATS

Study	Chemical	% Cl by Wt.	Strain	Sex	Dosage levels	Duration of Exposure*
Kimbrough, et al., 1975	Aroclor 1260	60%	Sherman	F	100 ppm	23 Months
Norback and Weltman, 1985	Aroclor 1260	60%	Sprague-Dawley	M/F	100 ppm for 16 months followed by 50 ppm for 8 months then control diet for 5 months	29 Months
Schaeffer, et al., 1984	Chlopen A 60	60%	Wistar	M	100 ppm	801-832 Days
NCI, 1977	Aroclor 1254	52-54%	F344	M/F	25 ppm, 50 ppm 100 ppm	104-105 Weeks
Schaeffer, et al., 1984	Clophen A 30	40-42%	Wistar	M	100 ppm	801-832 Days
Linder, et al., 1974 (Reproduction Study)	Aroclor 1260	60%	Sherman	M/F	100 ppm	9 Months

*Duration of exposure published by original investigator.

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METHODS

After assembling the hematoxylin and eosin stained microscope slides and data from each of the studies to be reviewed, the procedures developed by the National Toxicology Program which utilizes a Pathology Working Group (PWG) were generally followed to conduct the review. The PWG members consisted of Veterinary Pathologists with extensive experience in the microscopic evaluation and interpretation of hepatic changes observed in bioassay studies conducted in rodents. The PWG's task was to confirm the incidence of hepatocellular neoplasms, resolve diagnostic discrepancies, validate treatment-related effects and discuss the biological significance of the potential effects present.

Prior to the PWG review, all slides within each individual study were randomized by animal number and then coded in ascending numerical order. The randomized slides were examined without knowledge of treatment group by the Reviewing Pathologist, Dr. Deborah Banas, Experimental Pathology Laboratories, Inc. The reviewing pathologist recorded all changes present in the liver sections including both neoplastic and nonneoplastic lesions. After microscopic examination, the data was decoded and presented by treatment group for interpretation of the results and preparation for the Pathology Working Group review.

The Pathology Working Group was chaired by Dr. Jerry F. Hardisty, Experimental Pathology Laboratories, Inc., who organized and presented the material to a panel of five board certified Veterinary

Pathologists. The PWG review was performed on May 29-31, 1991 in the Research Triangle Park, North Carolina. Individuals attending or participating in the PWG review are listed as follows:

Dr. W. Ray Brown	(PWG Participant)
Dr. E.E. McConnell	(PWG Participant)
Dr. James A. Popp	(PWG Participant)
Dr. Robert A. Squire	(PWG Participant)
Dr. Jerrold M. Ward	(PWG Participant)
Dr. Deborah A. Banas	(Reviewing Pathologist)
Dr. Jerry F. Hardisty	(PWG Chairperson)
Dr. Ronald Moch	(Observer, FDA)
Dr. D. Singh	(Observer, EPA)
Dr. Jack Moore	(Observer, IEHR)
Dr. Renate Kimbrough	(Observer, IEHR)
Dr. W. Goessner	(Observer, GSF)
Dr. Diane Norback	(Observer, University of Wisconsin)
Dr. William M. Busey	(Observer, EPL)

Each participant recorded his diagnoses and comments on worksheets which were prepared by the PWG Chairman. The worksheets for each participant are on file at EPL. Each lesion was discussed by the group, reexamined if necessary and the final opinions were recorded on the Chairperson's Worksheets also maintained on file at EPL, Inc. The consensus diagnoses of the PWG was reached when at least three of five PWG participants were in agreement.

After the PWG completed the slide review for each study and the diagnoses recorded by the PWG Chairperson, the slides were decoded by treatment group. The consensus diagnoses was entered into a

computerized pathology reporting system at EPL, and Histopathology Incidence Tables for individual animals and Summary Incidence Tables were prepared for evaluation and interpretation of the PWG findings. The Histopathology Incidence Tables and Summary Incidence Tables are presented in Appendices A - E for each of the studies reviewed.

In addition to providing specific diagnoses for neoplastic and hyperplastic hepatocellular lesions present on the slides examined, the PWG participants also provided comments and opinions on the presence of a variety of nonneoplastic changes including foci of cellular alteration, and other lesions which may be indicative of potential hepatotoxicity. The PWG members were consistently in agreement with the reviewing pathologist concerning the nonneoplastic changes present. Since the reviewing pathologist had examined all liver slides in both control and treated animals in these studies using consistent criteria for diagnosis and severity grading, there was agreement by the PWG members to accept the reviewing pathologist's findings for nonneoplastic changes as the "consensus diagnosis". Therefore the consensus diagnoses presented in the Histopathology Incidence Tables and Summary Incidence Tables represent the majority opinion of the PWG for all neoplastic and hyperplastic changes and the reviewing pathologist's diagnosis for other nonneoplastic changes.

The diagnostic criteria used by the PWG participants and the reviewing pathologist for diagnosis of foci of cellular alteration,

hepatocellular hyperplasia and hepatocellular neoplasms is summarized below:

Foci of Cellular Alteration

1. Localized lesions recognized by virtue of tinctorial staining variation from surrounding hepatic parenchyma.
2. Usually do not compress but merge imperceptibly with surrounding parenchyma.
3. Minimal disruption of hepatic lobular architecture.
4. Hepatocytes within focus may have clear, eosinophilic, or basophilic cytoplasm or a mixture of these and may be larger or smaller than surrounding hepatocytes.

Focal Hepatocellular Hyperplasia

1. Usually a multifocal nodular lesion found associated with previous or concurrent hepatic damage. In this context, may be considered as multifocal regenerative hyperplasia.
2. Lesion consists of spherical proliferation of hepatocytes without nuclear atypia. May contain cytologic alterations similar to those seen in foci of cellular alteration.
3. An increased number of mitoses may be evident. Hyperplastic cells may be hypertrophic or contain intracytoplasmic vacuoles. The cells within a hyperplastic focus are usually uniform and have a homogeneous growth pattern.

4. Hepatic lobular architecture is evident but may be distorted. Portal triads can often be found within hyperplastic foci.

Hepatocellular Adenoma

1. Nodular proliferations of hepatocytes which are sharply demarcated by definite compression of surrounding hepatic parenchyma and usually by virtue of tinctorial staining differences.
2. Hepatic plates of an adenoma are usually not continuous with surrounding liver plates but impinge with them at a sharp angle.
3. Loss of normal lobular architecture.
4. Often have an increased mitotic index, may contain areas of cellular atypia, and have an irregular growth pattern.
5. Cells within an adenoma may be degenerative, hypertrophic, and/or contain intracytoplasmic vacuoles.

Hepatocellular Carcinoma

1. Usually considerably larger and more irregular than hepatocellular adenoma.
2. Compress or extend into surrounding hepatic parenchyma.
3. Characterized by one or more of the following: cellular atypia, local invasiveness, haphazardly arranged cells, broad sheets of cells, "trabecular" patterns, gland-like formations.

RESULTS

A. Aroclor 1260 in Female Sherman Rats (Kimbrough, et al., 1975)

Only one control female rat had a hepatocellular neoplasm. Control female 8231 had a hepatocellular carcinoma. In treated female rats, 21 rats had hepatocellular carcinomas and 135 rats had hepatocellular adenomas. A few of the treated female rats had both hepatocellular adenomas and hepatocellular carcinomas. The hepatocellular carcinomas were well-differentiated trabecular types. The hepatocytes varied from a normal appearance to enlarged, acidophilic or diffusely basophilic cells. The cytoplasm often contained eosinophilic inclusions within vacuoles. Hepatocellular adenomas were generally spherical and well demarcated. The cells in the adenomas were generally enlarged with nuclear and cytoplasmic features similar to the carcinomas. In three of the treated rats the liver tumors had glandular, papillary patterns giving the appearance of both hepatocytic and biliary epithelium (8326, 8377 and 8406). These tumors were considered to represent a subtype of hepatocellular adenoma or carcinoma and were not given a unique diagnosis. A summary of the incidence of rats with only hepatocellular adenoma and those with at least one hepatocellular carcinoma is presented as follows:

	Control	Aroclor 1260
No. Examined	187	189
No. Animals with only Hepatocellular Adenoma	0 (0%)	117 (61.9%)
No. Animals with at Least one Hepatocellular Carcinoma	1 (0.5%)	21 (11.1%)
Total Animals with Hepatocellular Neoplasms	1 (0.5%)	138 (73.0%)

Eosinophilic foci were present in 173 of the treated rats and only seven of the control rats. Other foci frequently occurred in addition to the eosinophilic foci in treated rats. The incidence of focus/foci of cellular alteration in the liver of control and treated female rats is presented as follows:

	Control	Aroclor 1260
No. Examined	187	189
Focus/Foci, Eosinophilic	7 (3.7%)	173 (91.5%)
Focus/Foci, Basophilic	4 (2.1%)	67 (35.4%)
Focus/Foci, Clear Cell	14 (7.5%)	67 (35.4%)
Focus/Foci, Mixed Cell	1 (0.5%)	38 (20.1%)
No. Animals with Any Type of Focus/Foci	25 (13.4%)	177 (93.7%)

Nonneoplastic lesions which appeared to be increased with treatment included centrilobular hepatocytomegaly, centrilobular fatty change,

bile duct hyperplasia, and pigment deposition. Oval cell proliferation, angiectasis, and dilated bile ducts were also increased in treated rats. Focal necrosis was often noted in treated rats in association with hepatocellular carcinomas. Cholangiofibrosis characterized by atypical bile ducts surrounded by abundant dense collagenous connective tissue was present in three treated rats. The incidence of nonneoplastic lesions which were increased in treated rats as compared to control rats is presented below:

	Control	Aroclor 1260
No. Examined	187	189
Centrilobular Hepatocytomegaly	1 (0.5%)	108 (57.1%)
Centrilobular Fatty Change	1 (0.5%)	32 (16.9%)
Bile Duct Hyperplasia	4 (2.1%)	29 (15.3%)
Pigment Deposition	9 (4.8%)	66 (34.9%)
Oval Cell Proliferation	2 (1.1%)	17 (9.0%)
Angiectasis	1 (0.5%)	17 (9.0%)
Dilated Bile Ducts, Focal	0 (0%)	14 (7.4%)
Focal Necrosis	0 (0%)	13 (6.9%)
Cholangiofibrosis	0 (0%)	3 (1.6%)

B. Reproduction Study With Aroclor 1260 in Male and Female Sherman Rats, F_0 Generation (Linder, et al., 1974)

Slight to severe centrilobular hepatocytomegaly was noted in all ten treated males, and minimal to moderately severe centrilobular hepatocytomegaly was present in seven of ten treated females. This lesion was characterized by enlarged hepatocytes, occasionally with atypical nuclei surrounding the central veins and, in the case of moderately severe or severe lesions, extending over a fair portion of the liver lobule. Cytoplasmic inclusions were prominent in these enlarged hepatocytes in two of the males. Minimal to slight centrilobular fatty change was noted in two of the treated females and focal fatty change occurred in one. Single eosinophilic foci were noted in two treated females. These lesions were characterized by foci of enlarged, eosinophilic hepatocytes having a ground-glass appearance to the cytoplasm and having a distinctly different tinctorial appearance compared to the surrounding parenchyma. Multiple, small, clear cell foci were noted in one treated male and were characterized by enlarged hepatocytes having a clear or slightly granular pale cytoplasm. Brown pigment deposition was noted in four of the treated females.

Other lesions noted in the liver sections of both control and treated rats were microgranulomas. These lesions consisted of focal accumulations of macrophages and mononuclear inflammatory cells, and

were unrelated to treatment with Aroclor 1260. The incidence of these changes are summarized as follows:

	Male Rats		Female Rats	
	Control	Aroclor 1260	Control	Aroclor 1260
No. Examined	10	10	10	10
Centrilobular Hepatocytomegaly	0 (0%)	10 (100%)	0 (0%)	7 (70%)
Focus/Foci, Eosinophilic	0 (0%)	0 (0%)	0 (0%)	2 (20%)
Focus/Foci, Clear Cell	0 (0%)	1 (10%)	0 (0%)	0 (0%)
Fatty Change, Centrilobular	0 (0%)	0 (0%)	0 (0%)	2 (20%)
Fatty Change, Focal	0 (0%)	0 (0%)	0 (0%)	1 (10%)
Cytoplasmic Inclusions	0 (0%)	2 (20%)	0 (0%)	0 (0%)
Microgranuloma(s)	3 (30%)	2 (20%)	5 (50%)	3 (30%)
Pigment Deposition	0 (0%)	0 (0%)	0 (0%)	4 (40%)

C. Aroclor 1260 in Male and Female Sprague-Dawley Rats (Norback and Weltman, 1985)

Hematoxylin and eosin stained slides were examined from 31 male control, 40 male treated, 45 female control and the 46 female treated Sprague-Dawley rats. Due to the fact that partial hepatectomies were performed on two control females (30 and 31) and two treated males (189 and 212), these animals are represented twice in the Histopathology Incidence Tables. The animal number followed by "A" represents the liver section examined following the partial hepatectomy and followed by "B" for the liver section examined at termination.

A marked sex difference in the number of hepatocellular neoplasms was present in this study. No hepatocellular neoplasms were present in any of the control male rats. In treated male rats four had

hepatocellular adenomas and one had a hepatocellular carcinoma. Only one control female rat had a hepatocellular adenoma. Hepatocellular adenomas were present in 29 treated female rats and hepatocellular carcinomas were present in 19 treated female rats. Additionally, three treated female rats had cholangiocarcinomas. Most of the hepatocellular carcinomas were well-differentiated trabecular neoplasms. Hepatocellular neoplasms in treated female rats 192, 212, 213 and 214 had glandular, papillary patterns similar to that described above for the Kimbrough study in female Sherman rats. Hepatocellular adenomas were generally spherical, well-demarcated tumors composed of well-differentiated cells which produced mild to moderate compression of the surrounding hepatic parenchyma. A few of the treated female rats had both hepatocellular adenoma and carcinoma. A summary of the incidence of male and female rats with only hepatocellular adenoma and rats with at least one hepatocellular carcinoma is presented as follows:

	Male Rats		Female Rats	
	Control	Aroclor 1260	Control	Aroclor 1260
No. Examined	31	40	45	46
No. Animals with only Hepatocellular Adenoma	0 (0%)	4 (10%)	1 (2.2%)	22* (47.8%)
No. Animals with at Least one Hepatocellular Carcinoma	0 (0%)	1 (2.5%)	0 (0%)	19* (41.3%)
Total Animals with Hepatocellular Neoplasms	0 (0%)	5 (12.5%)	1 (2.2%)	41 (89.1%)

*Two female rats with hepatocellular adenoma and one with hepatocellular carcinoma also had a cholangiocarcinoma.

An increased incidence of eosinophilic cell foci was present in treated male and female rats as compared to control rats. Although a

few basophilic, clear cell and mixed cell foci were also observed they did not occur in a treatment-related manner. A summary of the incidence of foci of cellular alteration in the liver in Sprague-Dawley Rats is presented below:

	Male Rats		Female Rats	
	Control	Aroclor 1260	Control	Aroclor 1260
No. Examined	31	40	45	46
Focus/Foci, Eosinophilic	1 (3.2%)	16 (40%)	5 (11.1%)	36 (78.3%)
Focus/Foci, Basophilic	1 (3.2%)	0 (0%)	2 (4.4%)	1 (2.2%)
Focus/Foci, Clear Cell	4 (12.9%)	0 (0%)	1 (2.2%)	0 (0%)
Focus/Foci, Mixed Cell	0 (0%)	2 (5%)	0 (0%)	0 (0%)
No. Animals with Any Type of Focus/Foci	5 (16.1%)	16 (40%)	7 (15.6%)	36 (78.3%)

Other hepatic lesions which appeared to be increased in treated groups as compared to control male and female rats included centrilobular hepatocytomegaly, centrilobular fatty change and centrilobular necrosis. In treated female rats the incidence of bile duct hyperplasia, cholangiofibrosis, cystic bile ducts, periportal fibrosis and pigment deposition was also increased in incidence as compared to control female rats. The incidence of selected hepatocellular lesions which were increased in incidence in treated male and/or female rats as compared to controls are presented as follows:

	Male Rats		Female Rats	
	Control	Aroclor 1260	Control	Aroclor 1260
No. Examined	31	40	45	46
Centrilobular Hepatocytomegaly	0 (0%)	15 (37.5%)	0 (0%)	5 (10.9%)
Centrilobular Fatty Change	0 (0%)	17 (42.5%)	0 (0%)	2 (4.3%)
Centrilobular Necrosis	0 (0%)	7 (17.5%)	2 (4.4%)	4 (8.7%)
Bile Duct Hyperplasia	6 (19.4%)	8 (20%)	2 (4.4%)	19 (41.3%)
Cholangiofibrosis	0 (0%)	0 (0%)	0 (0%)	4 (8.7%)
Cystic Bile Ducts	0 (0%)	1 (2.5%)	1 (2.2%)	5 (10.9%)
Periportal Fibrosis	2 (6.5%)	3 (7.5%)	0 (0%)	12 (26.1%)
Pigment Deposition	1 (3.2%)	2 (5.0%)	2 (4.4%)	13 (28.3%)

D. Clophen A 60 in Male Wistar Rats (Schaeffer, et al., 1984)

Hepatocellular adenomas were present in six control males and in 85 treated males. Hepatocellular carcinomas were present in two control males and in 67 treated males. Additionally one of the control males with a hepatocellular carcinoma also had a cholangiocarcinoma in the liver. Although most of the hepatocellular neoplasms consisted of well-differentiated adenomas and carcinomas, nine of the tumors present in the treated group had glandular, papillary patterns suggestive of a mixture of hepatocellular and biliary epithelium. Although this pattern was observed only in treated rats, it was considered to most likely represent a subclassification of hepatocellular adenoma or carcinoma and was not given a unique morphologic diagnosis. A few of the treated male rats had both hepatocellular adenomas and hepatocellular carcinomas. A summary of the incidence of male Wistar rats with only hepatocellular

adenoma and rats with at least one hepatocellular carcinoma is presented as follows:

	Control	Clophen A 60
No. Examined	120	125
No. Animals with only Hepatocellular Adenoma	6 (5.0%)	47 (37.6%)
No. Animals with at Least one Hepatocellular Carcinoma	2*(1.7%)	67 (53.6%)
Total Animals with Hepatocellular Neoplasms	8 (6.7%)	114 (91.2%)

*One control male also had cholangiocarcinoma.

Eosinophilic foci were present in the liver of 51 control and 101 treated rats. Additionally, the number of male rats with clear cell and mixed cell foci were greater in the treated group than in the control group. The incidence of focus/foci of cellular alteration in the liver of control and treated rats is presented as follows:

	Control	Clophen A 60
No. Examined	120	125
Focus/Foci, Eosinophilic	51 (42.5%)	101 (80.8%)
Focus/Foci, Basophilic	2 (1.7%)	4 (3.2%)
Focus/Foci, Clear Cell	7 (5.8%)	28 (22.4%)
Focus/Foci, Mixed Cell	7 (5.8%)	28 (22.4%)
No. Animals with Any Type of Focus/Foci	55 (45.8%)	108 (86.4%)

With the exception of an increased incidence of focal necrosis which was associated with hepatocellular carcinomas in rats receiving Clophen a 60, other nonneoplastic lesions appeared to be comparable or decreased in treated rats.

E. Clophen A 30 in Male Wistar Rats (Schaeffer, et al., 1984)

The Clophen A 30 study in Wistar rats was conducted at the same laboratory at the same time as the Clophen A 60 study discussed above and therefore shared the same control group. In the Clophen A 30 treated rats 14 hepatocellular adenomas and two hepatocellular carcinomas were present as compared to six adenomas and two carcinomas in the control group. All of the hepatocellular tumors in the control and treated rats occurred as singular tumors and none of the rats had both a hepatocellular adenoma and a hepatocellular carcinoma. One control male had a cholangiocarcinoma in addition to a hepatocellular carcinoma. A summary of the incidence of male rats with hepatocellular neoplasms is presented below:

	Control	Clophen A 30
No. Examined	120	128
Hepatocellular Adenoma	6 (5.0%)	14 (10.9%)
Hepatocellular Carcinoma	2 (1.7%)	2 (1.6%)
Total Hepatocellular Neoplasms	8 (6.7%)	16 (12.5%)

The incidences of focus/foci of cellular alteration of all types (basophilic, eosinophilic, clear cell and mixed cell) were greater in treated male rats than in control male rats. The incidence of focus/foci of cellular alterations present in control and treated groups is summarized as follows:

	Control	Clophen A 30
No. Examined	120	128
Focus/Foci, Eosinophilic	51 (42.5%)	98 (76.6%)
Focus/Foci, Basophilic	2 (1.7%)	15 (11.7%)
Focus/Foci, Clear Cell	7 (5.8%)	39 (30.5%)
Focus/Foci, Mixed Cell	7 (5.8%)	49 (38.3%)
No. Animals with Any Type of Focus/Foci	55 (45.8%)	106 (82.8%)

The incidence of other nonneoplastic lesions were either decreased or unchanged in the treated group as compared to the control group.

F. Aroclor 1254 in Male and Female Fischer 344 Rats (NCI, 1977)

The results of the PWG review generally confirmed the conclusion of the NCI Technical Report that "under the conditions of the bioassay Aroclor 1254 was not carcinogenic in Fischer 344 rats at the doses tested; however, a high incidence of hepatocellular proliferative lesions in both male and female rats was related to administration of the chemical".

nodular hyperplasia in both the male and female animals. There was one carcinoma and four adenocarcinomas in the gastrointestinal tract of treated rats. These neoplastic lesions are seen only sporadically and at a low incidence in the Fischer 344 rat; in this study no lesions of these types were diagnosed in either the male or female controls.

D. Statistical Analyses of Results

Tables C1 and C2 in Appendix C contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% of one or more treated groups.

In male rats, the results of the Cochran-Armitage test for positive dose-related trend in the incidences of leukemia and of combined leukemia and lymphoma are significant (P = 0.022 and P = 0.009, respectively). The corresponding results of the Fisher exact test, however, are not significant in any treated group when compared with the controls. There is no other incidence of tumors at any specific site in either sex which is statistically significant. A significant Cochran-Armitage trend in the negative direction is observed in the incidence of interstitial-cell tumor of the testis, where the incidence in the controls exceeds those in the mid- and high-dose groups.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by Aroclor® 1254, which could not be detected under the conditions of this test.

IV. DISCUSSION

At the doses used in this bioassay, Aroclor® 1254 was toxic to both male and female Fischer 344 rats, as shown by the dose-related depression of mean body weights and the clinical signs which occurred during the second year. Mean body weights of mid- and high-dose males and of all treated females were consistently lower than those of the corresponding controls after the initial growth phase. An intercurrent respiratory infection at week 30 resulted in temporary weight loss, but no deaths, in all groups including the controls; the animals later recovered without treatment. Clinical signs including alopecia, amber-colored urine, facial edema, exophthalmos, and cyanosis occurred in the high-dose groups beginning at week 72 and in the mid-dose groups at week 104. Survival among males, but not among females, showed a significant dose-related trend. Adequate numbers of animals of both sexes survived for meaningful statistical analyses of the incidences of tumors.

The combined incidences of lymphoma and leukemia in males were significant (controls 3/24, low-dose 2/24, mid-dose 5/24, high-dose 9/24, P = 0.009), using the Cochran-Armitage test for positive dose-related trend, but not in females (controls 4/24, low-dose 6/24, mid-dose 6/24, high-dose 6/24). Since the results of the Fisher exact test for increased incidence were not

significant for any of these groups, the occurrence of these lesions cannot clearly be related to the administration of Aroclor® 1254.

Hepatocellular changes including hyperplastic nodules, adenomas, and carcinomas were found in treated animals, but none of these lesions were found in control animals in this study. Hepatocellular carcinomas were observed in one mid-dose and two high-dose males, and hepatocellular adenomas were observed in one high-dose male, one mid-dose female, and two high-dose females. Nodular hyperplasia was diagnosed with a dose-related frequency in the low-, mid-, and high-dose male and female rats. Although the incidences of the tumors were not significant, the occurrence of these proliferative lesions appeared to be related to treatment.

In the stomach, jejunum, or cecum, adenocarcinomas were observed in two treated males and in two treated females as well as a carcinoma in one treated male. None of these lesions was found in control animals in this study, suggesting that the lesions -- although not statistically significant -- may be related to the administration of Aroclor® 1254.

The toxicity of polychlorinated biphenyls (PCBs) has been reviewed by several groups, including the Environmental

Protection Agency (1976), National Research Council (1976), Panel on Hazardous Trace Substances (1972), and International Agency for Research on Cancer (1974). Kimbrough et al. (1972) demonstrated hepatic adenofibrosis in male and female Sherman rats fed Aroclor® 1254 at up to 500 ppm for 8 months. A similar PCB, Kanechlor® 500, fed for 12 months to male Wistar rats, induced nodular hyperplasia at doses of 100-1,000 ppm; at 1,000 ppm, cholangiofibrosis also was induced (Ito et al., 1974). Keplinger et al. (1971; see also EPA Critera Document PCBs, 1976) fed Charles River rats up to 100 ppm Aroclor® 1254 for 24 months and reported originally that there was no significant increase in hepatic tumors in this study; re-evaluation of the liver slides, however, indicated a significant incidence of nodular hyperplasia in treated rats, compared with controls. Ito et al. (1973) observed nodular hyperplasia and well-differentiated hepatocellular carcinoma in male strain dd mice fed 500 ppm Kanechlor® 500 for 8 months, and Kimbrough and Linder (1974) observed adenofibrosis and hepatomas in BALB/cJ mice fed 300 ppm Aroclor® 1254 for 11 months.

In a study of a closely related PCB, Kimbrough et al. (1975) observed hepatocellular carcinomas in female Sherman rats fed 100 ppm Aroclor® 1260 for 21 months.

It is concluded that under the conditions of this bioassay,

Aroclor® 1254 was not carcinogenic in Fischer 344 rats; however, a high incidence of hepatocellular proliferative lesions in both male and female rats was related to treatment. In addition, the carcinomas of the gastrointestinal tract may be associated with treatment in both males and females.

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Polychlorinated Biphenyl Induction of Hepatocellular Carcinoma in the Sprague-Dawley Rat

by D. H. Norback* and Robert H. Weltman*†

Male and female Sprague-Dawley rats (70 males and 70 females in the initial group) were fed a diet containing a polychlorinated biphenyl mixture (Aroclor 1260, 100 ppm for 16 months and 50 ppm for an additional 8 months) for 2 years followed by a control diet for 5 months. A control group initially consisted of 63 males and 63 females. Sequential morphologic changes were evaluated throughout the study. In the PCB-exposed group the following hepatocellular lesions developed in sequence: centrolobular cell hypertrophy at 1 month, foci of cell alteration at 3 months, areas at 6 months, neoplastic nodules at 12 months, trabecular carcinoma at 15 months, and adenocarcinoma at 24 months. In addition, simple and cystic cholangioma at 18 and 23 months, respectively, and adenofibrosis at 22 months were present. With the exception of hepatocyte hypertrophy and adenofibrosis, all lesions contained cells that were positive for gamma glutamyl transpeptidase activity. In the PCB-exposed group that was examined after 18 months, hepatocellular neoplasms were present in 95% of the 47 females and in 15% of the 46 males. Distant organ metastases did not occur and the mortality rate was not increased in the PCB exposed group. In 81 control rats examined after the 18th month, only 1 hepatocellular neoplasm (a neoplastic nodule) occurred. PCB-exposed and control rats developed simple cholangioma, cystic cholangioma and adenofibrosis; the incidence of each was greater in the PCB group. Thus, within the Sprague-Dawley rat group exposed to a diet with relatively high concentrations of Aroclor 1260 for 2 years a hepatocarcinogenic effect manifested by formation of slowly growing hepatocellular carcinomas was produced. The incidence of hepatocellular neoplasms in females was strikingly greater than in males.

Introduction

Polychlorinated biphenyl (PCB) mixtures have produced a variety of oncogenic effects in the rat liver. Adenofibrosis developed in male and female Sherman rats which received a diet containing 500 μg Aroclor 1254/g for 30 weeks (*1*), in female Sherman rats which received a diet containing 100 μg Aroclor 1260/g for 21 months (*2*), and in male Wistar rats which received a diet containing 1 mg Kanechlor 500, 400 or 300/g for 40 to 52 weeks (*3*). Foci and areas of hepatocyte alteration and neoplastic nodules developed in female Sherman rats which received 100 μg Aroclor 1260/g diet for 21 months (*2*). Neoplastic nodules also developed in female, but not male, Donryo rats which received a diet containing Kanechlor 400 ranging in concentration from 33.5 to 616 μg/g for 400 days (*4*) and in male Wistar rats which received a diet containing 100 μg Kanechlor 500, 400 or 300/g for 40 to 52 weeks (*3*). Hepatocellular carcinoma developed in female Sherman rats which received 100 μg Aroclor 1260/g diet for 21 months (*2*). Liver lesions designated as hepatomas by the investigator occurred in albino rats which received 100 μg of Aroclor 1242, 1245 or 1260/g diet for 24 months (*5*).

We investigated the hepatocarcinogenic potential of the highly chlorinated PCB mixture Aroclor 1260 in another strain of rat, the Sprague-Dawley, which has a low incidence of spontaneous hepatocellular neoplasms (*6*), Enzyme histochemistry and other morphologic studies further characterized the lesions. The study, which spanned the natural life of the animal, allowed us to further evaluate the potential of the hepatocellular carcinoma to metastasize to distant organs and the effect of PCBs on longevity of the animal. Morphologic studies of the liver throughout the course of the experiment permitted evaluation of the sequential development of the liver lesions. The incidence of tumors occurring in male and female rats was determined.

Materials and Methods

Weanling Sprague-Dawley rats, initially weighing 100 gm, were divided into two groups. The PCB-treated group, initially containing 70 males and 70 females, re-

*University of Wisconsin, Clinical Laboratories, Department of Pathology and Laboratory Medicine, Madison, WI 53792.
†Present address: Hazleton Laboratories America Inc., P.O. Box 7545, Madison, WI 53707.

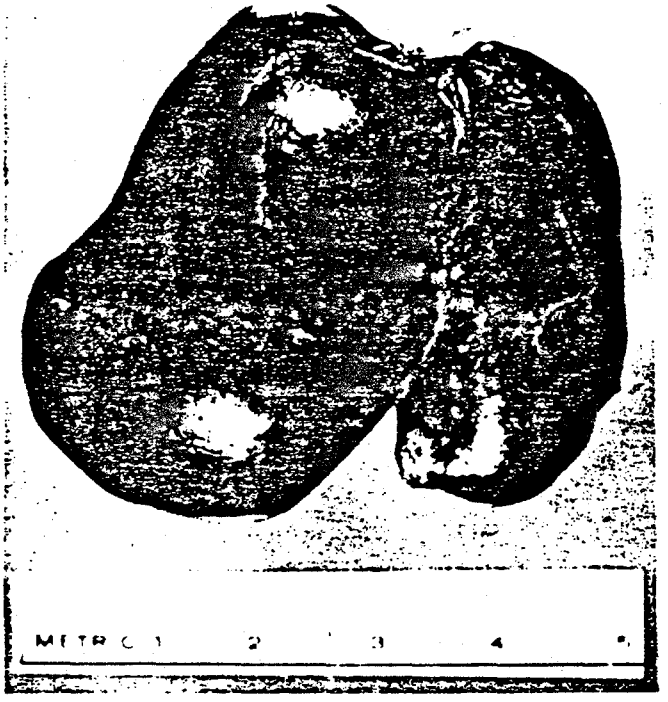


FIGURE 1. PCB-exposed rat liver; at 23 months. The liver surface is dotted with nonelevated tan foci, 0.5 to 1 mm in diameter. A neoplastic nodule is present at the tip of one lobe.

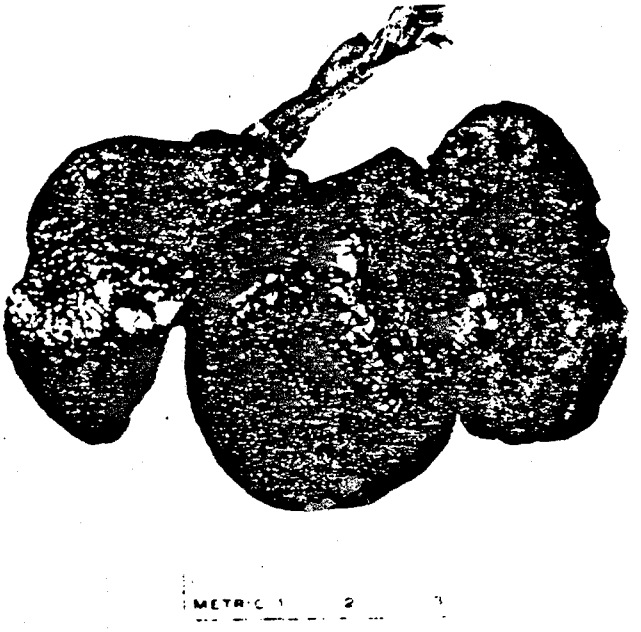


FIGURE 2. PCB-exposed rat liver; at 27 months. One lobe (cut surface) is replaced by hepatocellular carcinoma with necrotic center. A neoplastic nodule protrudes from another lobe. Small tan foci are numerous.

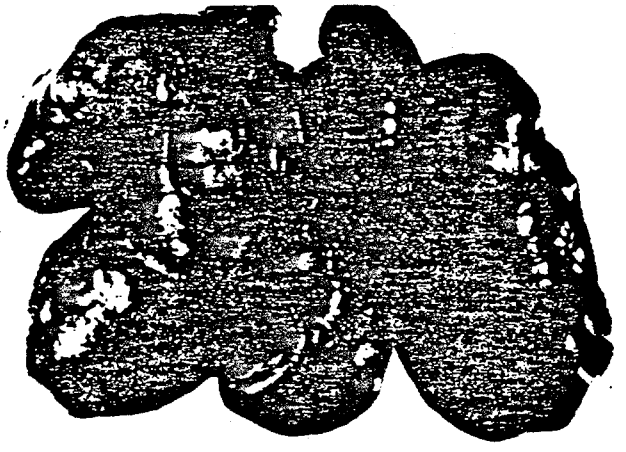


FIGURE 3. PCB-exposed rat liver; the liver lobe (at left) is replaced by adenocarcinoma which appeared as a tan tumor with cysts at the 29th month. Adenofibrosis is observed as a firm white lesion with central depression.

ceived a basal diet (Purina Rat Chow, St. Louis, MO) with added Aroclor 1260 (Monsanto Chemical Co., St. Louis, MO) at a concentration of 100 μg/g diet for 16 months and 50 μg/g diet for an additional 8 months. The diet was prepared by mixing Aroclor 1260 with corn oil, adding the mixture to ground chow, and pelleting the final mixture. The control group, initially containing 63 males and 63 females, received the basal diet with added corn oil for 18 months and the basal diet alone for an additional 5 months. All surviving rats received the basal diet from the 25th month to the 29th month. Both groups received water *ad libitum*. After a 24-hr fast, the medial and left lobes of the liver of ten etherized rats (two male controls, two female controls, three male PCB-treated, and three female PCB-treated rats for each time period) were removed at 1, 3, 6, 9, 12, 15 and 18 months. Partial hepatectomy was performed once per animal in these groups. At 24 months a similar group and at 29 months all remaining animals were sacrificed. Throughout the study moribund rats were sacrificed. At death all rats were necropsied. Liver weights and body weights were recorded. Representative slices from all liver tissue obtained at surgery and at necropsy, and slices from other selected organs obtained at necropsy, were prepared for microscopy. Tissue slices were placed in a formaldehyde fixative, dehydrated in ethanol, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H + E) or periodic acid–Schiff (PAS) stain. Liver tissue was also diced into 1 mm cubes, fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.4–7.5) for 4 to 24 hr, rinsed with buffer, post-fixed in 1% osmium tetroxide buffered with 0.1 M veronal acetate (pH 7.4) for 30 min, dehydrated in ethanol, infiltrated with propylene oxide and then Epon-Araldite, sectioned at 1 to 2 μm and stained with Toluidine Blue (TB). Between 9 and 29 months, liver slices from at least two animals of each group at each examination time point were frozen on dry ice and processed for γ-glutamyl transpeptidase (GGT) activity according to the procedure of Rutenburg et al. (7).

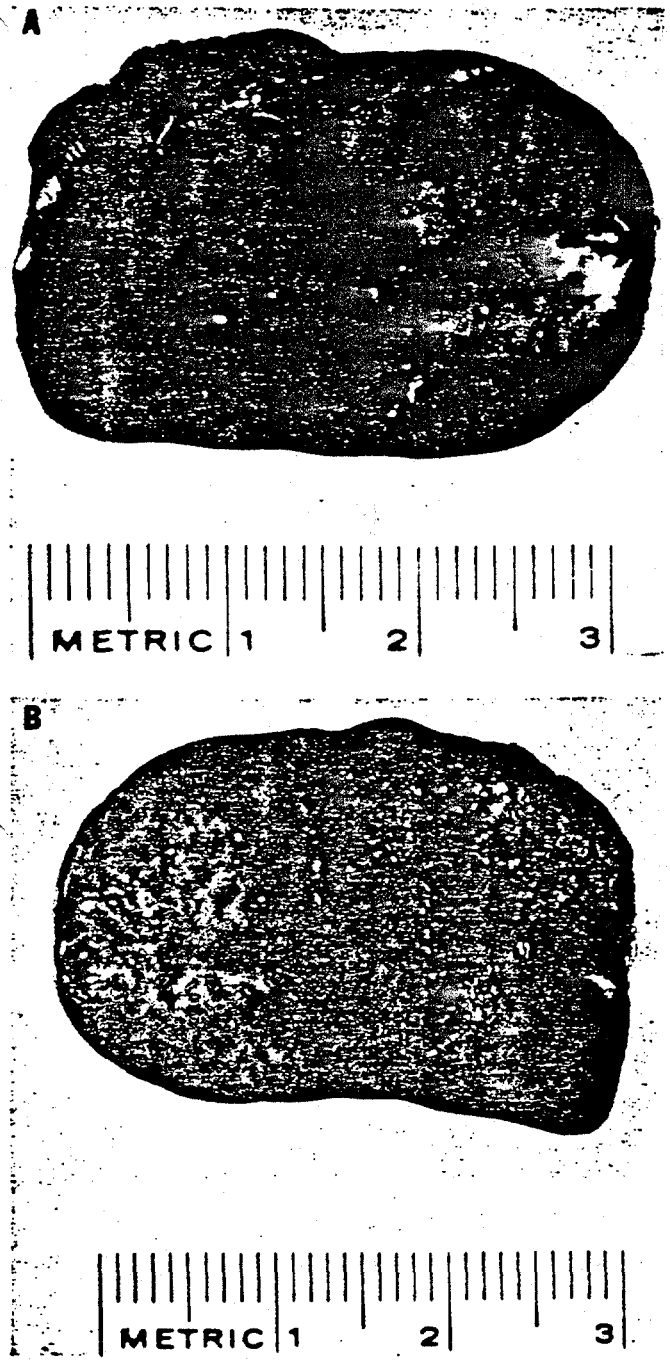


FIGURE 4. PCB-exposed rat liver; (*A*) at 24 months, prominent vasculature and cystic spaces of this hepatocellular carcinoma are evident through the hepatic capsule; (*B*) the cut surface shows irregular borders and extensive replacement of the lobe.

Results

Macroscopic

Chronic dietary administration of Aroclor 1260 caused early and progressive liver alterations in the Sprague-Dawley rats. Hepatomegaly was apparent during surgery at the first month, and after 18 months the livers

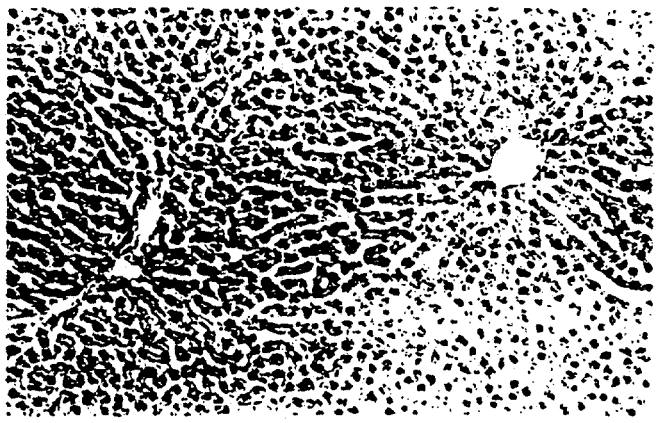


FIGURE 5. Control rat liver, a central vein, portal triads, thin plates of uniform hepatocytes, and endothelial-lined sinusoids were structures of the normal liver as demonstrated from a rat fed the control diet for 9 months. H & E; ×160.

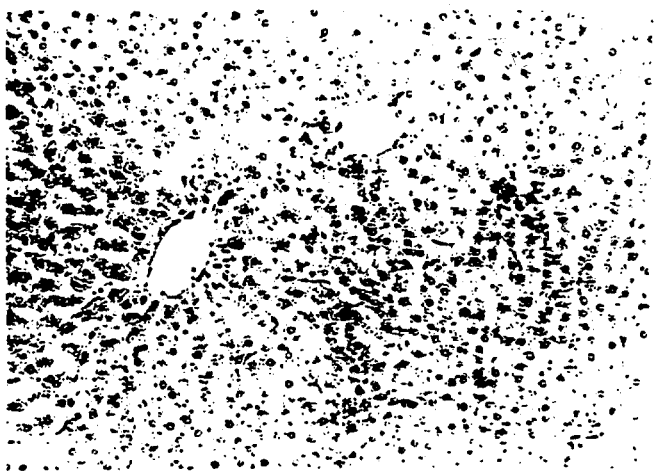


FIGURE 6. Hypertrophic hepatocytes developed in the central lobular region of the liver obtained at 1 month. H & E; ×160.

of female rats averaged 12% of the body weight, while the control averaged 4%. Small (1 mm) tan areas (Fig. 1) were readily apparent on the capsular surface. Neoplastic nodules (Figs. 1 and 2) near the capsular surface protruded and compressed the surrounding parenchyma. Hepatocellular carcinoma (Figs. 2–4) often replaced the major portion of the lobe and elevated the liver surface. The size ranged from 0.5 to 6.0 cm. Surface vessels were often apparent through the capsule. The ill-defined borders compressed the adjacent parenchyma. Portions of the tumor were hemorrhagic or necrotic. Some tumors contained cystic areas with clear fluid (Fig. 3). Adenofibrosis (Fig. 3) appeared as a firm white area with central depression. In some livers, all lesions were present.

Microscopic

The hepatocellular lesions were classified according to recommendations of Stewart et al. (*8*). For the cholangiocellular lesions,the nomenclature of Schauer and

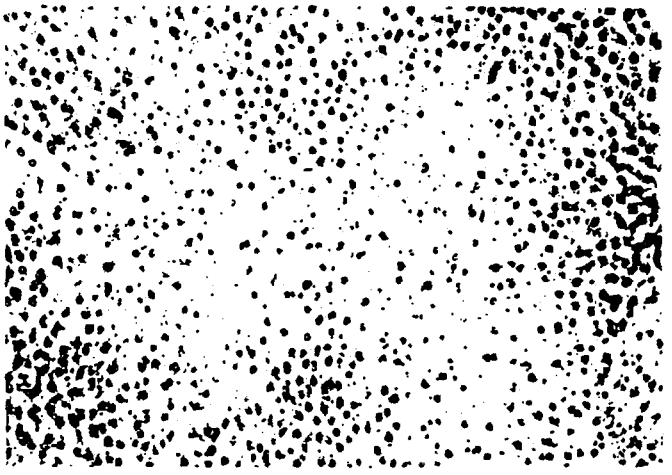


FIGURE 7. Foci of altered cells developed in the central and middle lobular regions in this liver obtained at 9 months. The cells merged with adjacent hepatic plates. Cells of the focus were usually eosinophilic and larger than normal. H & E; ×150.

FIGURE 8. This enzyme altered focus was present at 9 months. GGT; ×160.

FIGURE 9. This neoplastic nodule in a liver obtained at 15 months lacked lobular architecture and compressed the adjacent nontumor parenchyma. H & E; ×40.

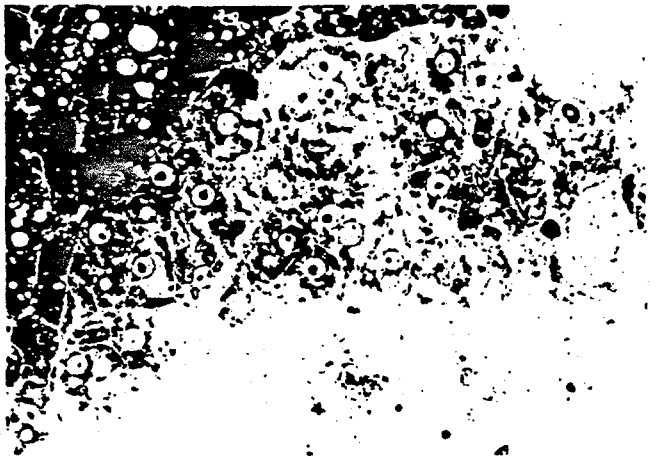


FIGURE 10. Cells, nuclei, and nucleoli in the neoplastic nodule described for Fig. 9 were larger than their counterparts of the adjacent parenchyma. In this preparation, the nucleus is light with a very dark nucleolus. The granularity of the cytoplasm is mainly due to mitochondria. TB; ×400.

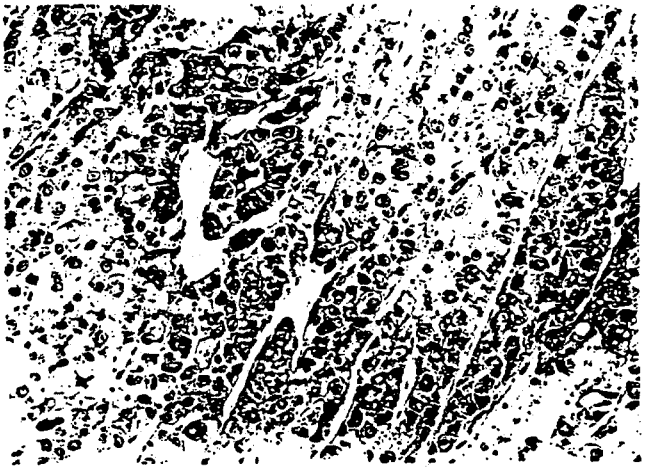


FIGURE 11. In a trabecular carcinoma from a liver obtained at 24 months, wide plates of cells were separated by sinusoids. The large cells contained large, abnormal nuclei. H & E; ×160.

Kunze (*9*) is applied. The normal architecture of the liver is shown in Figure 5. In the PCB-exposed group, the following hepatocellular lesions were observed in sequence (Table 1): centrolobular cell hypertrophy (Fig. 6) at 1 month, foci (Figs. 7 and 8) at 3 months and then areas of cell alteration at 6 months, neoplastic nodules (Figs. 9 and 10) at 12 months, trabecular carcinoma (Figs. 11 and 12) at 15 months, and adenocarcinoma (Fig. 13) at 24 months. In addition, simple (Fig. 14) and cystic (Fig. 15) cholangioma at 18 and 23 months, respectively, and adenofibrosis (Fig. 16) at 22 months were present.

There was no evidence of metastasis to the lung by gross or microscopic examination.

Control livers and livers containing all lesions were evaluated for GGT activity. Throughout the study, GGT positive areas of hepatocytes were absent in control

Table 1. Development of preneoplastic and neoplastic heptatocellular lesions in male and female rats during chronic Aroclor 1260 exposure.[a]

	No. of livers with lesions of each three sampled															
	1 mo.		3 mo.		6 mo.		9 mo.		12 mo.		15 mo.		18 mo.		24 mo.	
Lesion	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Focus	0[2]	0	2	2	3	3	3	3	3	3	3	3	3	3	3	3
Area	0	0	0	0	1	0	2	1	0	3	1	3	0	3	3	2
Neoplastic nodule	0	0	0	0	0	0	0	0	0	1	0	3	0	3	1	3
Trabecular carcinoma	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	2
Adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

[a]These lesions were not present in sequentially sampled control liver.

Table 2. Incidence of Aroclor 1260-exposed and control animals containing hepatocellular neoplasms.

	Incidence in Aroclor 1260 animals, %[a]			Incidence in control animals, %[a]		
	Total (N = 93)	Male (N = 46)[b]	Female (N = 47)[c]	Total (N = 81)	Male (N = 32)[d]	Female (N = 49)[e]
Trabecular carcinoma[f]	23(21)	4(2)	40(19)	0	0	0
Adenocarcinoma[f,g]	26(24)	0	51(24)	0	0	0
Neoplastic nodule only	8(7)	11(5)	4(2)	1(1)	0	2(1)
Negative	44(41)	85(39)	4(2)	99(80)	100	98(48)

[a]Figures in parentheses denote number of animals which survived 18 mo. or longer.
[b]Includes eight animals that had received a partial hepatectomy during the first 18 mo.
[c]Includes seven animals that had received a partial hepatectomy during the first 18 mo.
[d]Includes eight animals that had received a partial hepatectomy during the first 18 mo.
[e]Includes ten animals that had received a partial hepatectomy during the first 18 mo.
[f]Animals containing neoplastic nodules plus carcinoma were only included in the carcinoma category.
[g]Animals with trabecular carcinoma and adenocarcinoma were only placed in adenocarcinoma category.

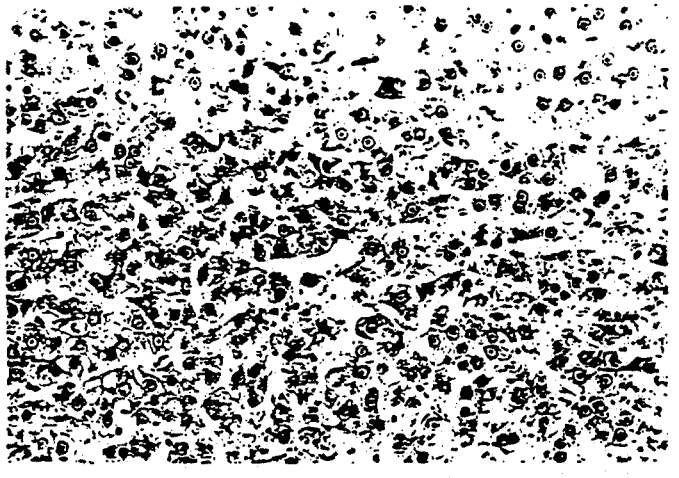


FIGURE 12. Some sections of trabecular carcinoma obtained at 24 months contained numerous mitoses. H & E; ×200.

rats. Hypertrophic cells of the center of the lobule were uniformly negative. Some foci of cell alteration were strongly positive (Fig. 8). Neoplastic nodules and hepatocellular carcinomas (13G) contained positive cells admixed with negative cells. The cells lining the luminal structure of adenocarcinoma were more strongly positive than the cells forming trabecular structures (13G). In the one adenofibrosis evaluated for GGT activity, the ductal cells were GGT negative while entrapped hepatocytes, bile duct cells, and cystic cholangioana cells were positive (Fig. 17).

All adenocarcinomas had elements of trabecular patterns of growth, and all trabecular carcinomas had cell arrangements that resembled a glandular, ductal, or cystic pattern. The apparent lumens of adenocarcinoma probably result from individual cell necrosis within a trabeculum, formation of large canalicularlike structures, cross sections of sinusoids, or glandlike formations formed from cells that differentiate toward the cuboidal or columnar morphology. Close association of hepatocytelike cells and ductal-type cells lining the cystic space, as well as the presence of cells with intermediate morphology (Fig. 13*F*), suggests a common origin of the cells in adenocarcinoma.

The percentage of animals with hepatocellular neoplasms occurring in animals that survived for 18 months or longer is presented in Table 2. Hepatocellular neoplasms were uncommon in control rats; only one (4 mm diameter) hepatocellular neoplasm occurred in 81 control animals examined. Hepatocellular neoplasms developed in over 55% of the 93 livers (7 males and 45 females) examined after the 18th month. Females had the highest incidence of heptocellular neoplasms; more than 95% developed tumors. Few males developed tumors; neoplastic nodules were present in 11% and hepatocellular carcinoma in 4%.

Treated and control rats developed cholangiocellular

Table 3. Incidence of Aroclor 1260-exposed and control animals containing cholangiocellular lesions.

	Incidence in Aroclor 1260 animals, %[a,b]			Incidence in control animals, %[c]		
	Total (N = 93)	Male (N = 46)[b]	Female (N = 47)	Total (N = 81)	Male (N = 32)[d]	Female (N = 49)[e]
Cholangioma (simple)	38(35)	30(14)	45(21)	5(4)	6(2)	4(2)
Cholangioma (cystic)[f]	8(7)	4(2)	10(5)	1(1)	0	2(1)
Adenofibrosis[g]	9(8)	2(1)	15(7)	4(3)	6(2)	2(1)
Negative	46(43)	63(29)	30(14)	90(73)	88(28)	92(45)

[a]Figures in parentheses denote number of animals which survived 18 mo. or longer.
[b]Includes eight animals that had received a partial hepatectomy during the first 18 mo.
[c]Includes seven animals that had received a partial hepatectomy during the first 18 mo.
[d]Includes eight animals that had received a partial hepatectomy during the first 18 mo.
[e]Includes ten animals that had received a partial hepatectomy during the first 18 mo.
[f]Animals contain simple cholangioma (but not adenofibrosis)
[g]Animals also containing cholangioma were placed in this group.

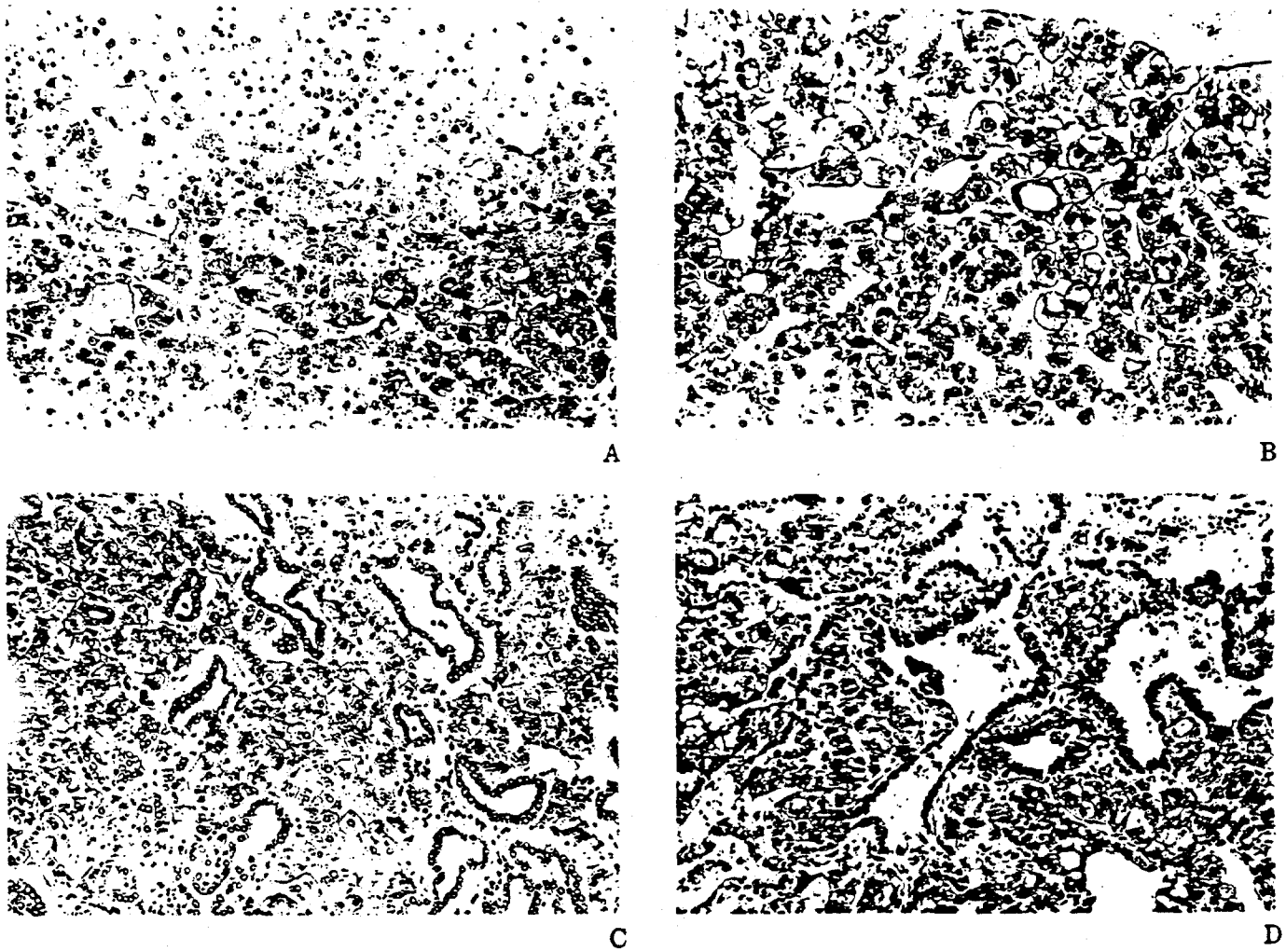
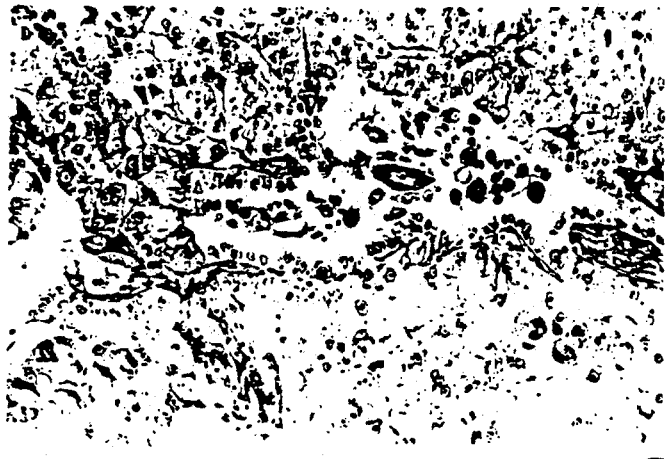


FIGURE 13. All tumors with adenocarcinoma pattern contained trabecular regions. The cells forming gland-, duct-, or cystlike structures appeared to be hepatocellular with unusual features and the luminal structures likely arose from several processes. (A) In this liver obtained at 24 months, some cystic spaces appeared to result from degeneration of individual cells within trabeculae; H & E, ×160. (B) In this liver obtained at 29 months, the glandular spaces represent exaggerated canaliculi formed by three to five hepatocytes. Occasionally, a cross section of a sinusoid may appear as a glandular lumen; however, the presence of endothelial cells should exclude this interpretation; H & E, ×180. (C) In this liver obtained at 29 months, cuboidal cells formed duct-or cyst-like structures among hepatocyte-type cells; H & E, ×160. (D) In this liver obtained at 29 months, columnar cells also lined duct-like structures and covered papillary projections; H & E, ×160.



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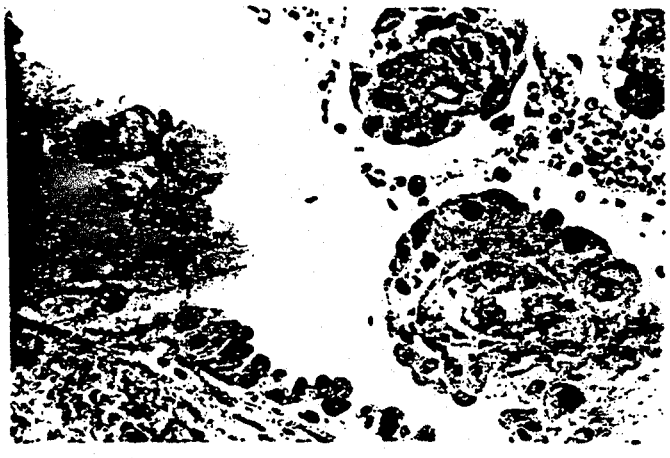

F

G

FIGURE 13 (con't). (E) In this liver obtained at 29 months, intracellular mucus was not demonstrated with special stains; PAS, ×180. (F) Cells lining ductlike structures with hepatocellular-type morphology were continuous with the cuboidal cells; TB, ×300. (G) In a carcinoma with trabecular and adeno- patterns, the lining cells are strongly GGT-positive, and the trabeculae show a variegated pattern; GGT, ×140.

lesions. The simple cholangioma, cystic cholangioma, and cholangiofibrosis of Schauer and Kunze (*9*) are referred to as bile duct hyperplasia, cyst, and adenofibrosis, respectively, by Stewart et al. (*8*). Although the cholangiocellular tumors occurred in the control rats, the incidence of each was greater in the PCB-treated group (Table 3).

Discussion

Hepatocarcinogenic activity of PCBs was demonstrated in the Spague-Dawley rat after long term exposure to relatively high dietary concentrations of Aroclor 1260. Large hepatocellular carcinomas, measuring up to 6 cm in diameter, nearly replaced the liver lobes. Histologic features of carcinoma included wide trabeculae formed from large hepatocytelike cells containing large abnormal nuclei with clumped peripheral chromatin and huge nucleoli. Some microscopic fields contained numerous mitotic figures. Central necrosis and hemorrhage were sometimes present. The tumors were not encapsulated and extended into the adjacent nontumorous parenchyma.

Although the tumors met the morphologic criteria for malignancy, their biologic behavior was relatively unaggressive. The neoplasms did not metastasize to distant organs nor invade blood vessels. Mortality of the animals was not increased. The lack of greater morbidity or mortality is likely due to slow progression of the neoplastic process and late appearance and slow growth of the hepatocellular carcinoma.

PCBs have been established as very effective promoters in carcinogenesis. Kanechlor 400 (400 μg/g diet given for 6 months) after 3'-methyl-4-dimethylaminoazobenzene increased the incidence of hepatocarcinoma over that for the initiator alone in female Donryo rats in the 800-day study (*10*). Kanechlor 500 (0.01 mL given twice weekly by gastric intubation for 12 weeks) resulted in liver tumors at 40 and 52 weeks in male Wistar rats (*11*). Kanechlor 500 (500 or 1000 μg/g diet) caused development of neoplastic nodules in male F344 rats when given for 8 weeks after a nontumorigenic dose of *N*-2-fluorenylacetamide (*12*). Aroclor 1254 (100 μg/g diet for 18 weeks) increased the incidence of hepatocellular carcinoma when given to male Sprague-Dawley rats after diethylnitrosamine (*13*). Aroclor 1254 (500 mg/kg body weight given by intraperitoneal injection) reduced the time required for the appearance of enzyme altered foci in partially hepatectomized male Sprague- Dawley rats (*14*).

It remains to be established whether PCBs also have an initiating effect or whether the neoplasms result from promotion of a background incidence of initiated cells. In hepatocarcinogenesis, it is difficult to distinguish a

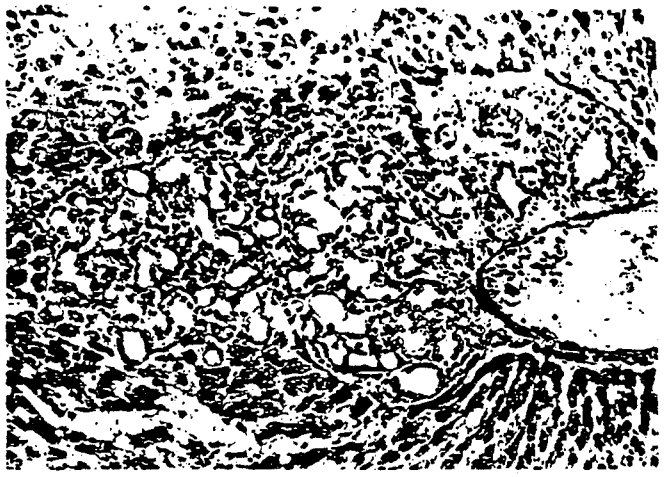

FIGURE 14. A simple cholangioma, a proliferation of bile ducts, occurred in a periportal region at 18 months. H & E; ×160.

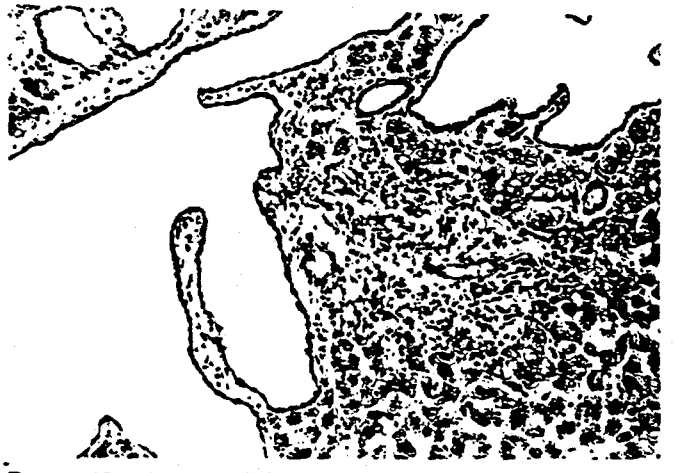

FIGURE 15. A cystic cholangioma, which does not compress surrounding parenchyma, was present at the 29th month. H & E; ×160.

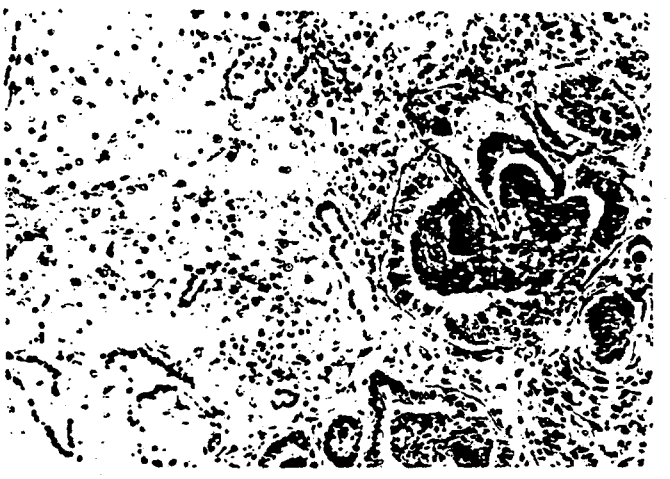

FIGURE 16. Adenofibrosis obtained at 24 months consisted of gland-like structures formed from columnar epithelium surrounded by abundant stroma. Some cells contained mucin. PAS; ×160.

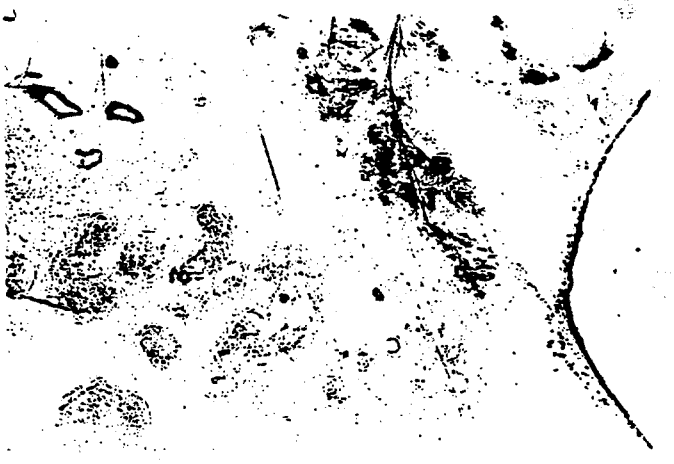

FIGURE 17. Glandular formations of adenofibrosis are negative for GGT activity, while cystic cholangioma and a focus of hepatocytes were positive. GGT; ×30.

weak initiator from a strong promoter (*15*). A possible mechanism of initiation by PCBs is the formation of an arene oxide of a PCB analog, which is an electrophilic intermediate metabolite capable of forming an adduct with DNA. The identification of a *trans*- dihydrodiol in the rat (*16*) supports the supposition that the PCBs are metabolized via arene oxides. Evidence against the ability of PCBs to act as an initiator resulted from mutagenic studies. While most initiators tested with the Salmonella/microsome test are detected as mutagens (*17*), a PCB mixture (Aroclor 1254) was not mutagenic in the Salmonella assay in the absence or presence of uninduced or PCB-induced rat liver homogenate (*18*).

Hepatocellular lesions developed in more than 95% of the Aroclor 1260 fed female rats, whereas male rats had a 15% incidence of hepatocellular neoplasms. Kimura and Baba (*4*) also noted a higher incidence of hepatic neoplasms in female than in male Donryo rats. Sex difference in the incidence of PCB-induced hepatocarcinogenesis may be related to sex-linked differences in enzymatic activation and deactivation of carcinogens as proposed for acetylaminofluorine hepatocarcinogenicity (*19*), or presence of androgens or estrogens which compete for the carcinogen for metabolism as proposed for benzopyrene (*20*), aflatoxin (*21*) or dimethylbenzanthracene (*22*) hepatocarcinogenicity.

Adenofibrosis consists of glandular structures lined by mucin-secreting columnar or cuboidal cells and surrounded by layers of connective tissue. An electron microscopic study identified the lesion as intestinal metaplasia with goblet cells, enterochromaffin cells, and Paneth cells (*23*). The lack of GGT activity in adenofibrosis also suggests the lesion is quite distinct from simple cholangioma or cholangiocarcinoma, both being strongly positive for GGT (*24*).

The GGT assays were performed in Henry Pitot's laboratory at McArdle Cancer Research Institute.

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Portions of this paper have been published in abstract form (*25–29*).

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Pathology of Chronic Polychlorinated Biphenyl (PCB) Feeding in Rats

EKKEHARD SCHAEFFER, HELMUT GREIM, AND WOLFGANG GOESSNER

Gesellschaft für Strahlen- und Umweltforschung mbH, Institut für Biologie, Abteilung für Pathologie, und Institut für Toxikologie und Biochemie, Abteilung für Toxikologie, Ingolstaedter Landstrasse 1, D-8042 Neuherberg, West Germany

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Pathology of Chronic Polychlorinated Biphenyl (PCB) Feeding in Rats. SCHAEFFER, E., GREIM, H. AND GOESSNER, W. (1984). *Toxicol Appl Pharmacol* 75, 278–288. The hepatocarcinogenic effect of Clophen A 30 and Clophen A 60 was tested in male weanling rats by long-term feeding over a period of 832 days. The mortality rate was investigated in 100-day intervals. In the first 800 days liver carcinoma accounted for 21% of necropsies in the Clophen A 60 group but only 2% of the necropsies in the Clophen A 30 group and none in the control animals. The tumors were first observed after 700 days. After 800 days hepatocellular carcinoma was the most common lesion observed in the Clophen A 60 animals (61%) whereas it was only observed in 3% of animals in the Clophen A 30 group and 2% in the controls. Preneoplastic lesions, such as foci of hepatocellular alterations and neoplastic nodules, were first observed after Day 500. The incidence of foci predominated in all time intervals, but an increase in neoplastic nodules and hepatocellular carcinomas was observed with increased time. There was a marked trend from foci to neoplastic nodule to hepatocellular carcinoma with time. The total mortality rate and the incidence of thymoma, inflammatory lesions of the urogenital tract, in the experiment were significantly reduced by Clophen administration. Whether this protective effect could be induced by polychlorinated biphenyls (PCBs) is discussed.

Polychlorinated biphenyls (PCBs) have been shown to produce foci of hepatocellular alterations (Kimbrough *et al.*, 1975), neoplastic nodules (Kimura and Baba, 1973; Ito *et al.*, 1974; Kimbrough *et al.*, 1975), and hepatocellular carcinomas (Kimbrough *et al.*, 1975) in rats, and hepatomas (Kimbrough and Linder, 1974; Nagasaki *et al.*, 1972; Ito *et al.*, 1973) and hepatocellular carcinomas (Ito *et al.*, 1973) in mice. Furthermore PCBs have been shown to have a promoting activity on enzyme-altered islands and neoplastic nodules in rats (Kimura *et al.*, 1976; Nishizumi, 1976; Tatematsu *et al.*, 1979; Preston *et al.*, 1981; Pereira *et al.*, 1982; Oesterle and Deml, 1983). Adenofibrosis in rats (Kimbrough *et al.*, 1972, 1973; Ito *et al.*, 1974) and in mice (Kimbrough and Linder, 1974) resulting from PCB consumption has also been observed.

In general hepatocellular carcinomas have been induced in rodents by highly chlorinated PCBs only. Feeding experiments over 224 days with Kanechlor 300, 400, and 500 in mice were performed by Ito *et al.* (1973). Hepatocellular carcinomas were induced by the highest chlorinated compound only (Kanechlor 500). In a similar experiment in rats (Ito *et al.*, 1974), different PCBs were administered for 364 days. Although no carcinomas were observed, the occurrence of cholangiofibrosis and nodular hyperplasia indicated that hepatocellular carcinomas could probably emerge after a longer administration period. Administration of the highly chlorinated PCB Aroclor 1260 to rats for 600 days resulted in hepatocellular carcinomas (Kimbrough *et al.*, 1975).

The present experiments were designed to test the hepatocarcinogenic effect of two different chlorinated commercial PCB mixtures,

0041-008X/84 $3.00

Clophen A 30 (30 wt% of chlorines) and Clophen A 60 (60 wt% of chlorines) in male rats when administered by continuous feeding over a period of more than 800 days, i.e., until the end of the experiment.

METHODS

A total of 432 male weanling, specific-pathogen-free, random-bred Wistar rats (Neuherberg, FRG) were used in the experiments. Animals were maintained at a room temperature of 22°C and humidity of 50 ± 5% and given feed (commercial stock diet Altromin (1324) supplemented as described below) and water *ad libitum*. For the first 8 weeks all rats received the standard diet. After 8 weeks the rats were divided into three groups. One hundred thirty-nine control animals (group 1) received the basic diet; 152 animals (group 2) received the basic diet supplemented with 100 ppm Clophen A 30; and 141 animals (group 3) received the basic diet supplemented with 100 ppm Clophen A 60.

The PCBs were the kind gift of Dr. Wrabetz, Bayer (Leverkusen, FRG) and added to the pellets by Altromin (Lage, FRG) during production. Clophen A 30 had an average composition of 1.0% monochlorbiphenyl, 20.7% dichlorbiphenyl, 57.4% trichlorbiphenyl, 17.3% tetrachlorbiphenyl, 1.8% pentachlorbiphenyl, 1.0% hexachlorbiphenyl, 0.6% heptachlorbiphenyl, and 0.1% octachlorbiphenyl; furans were absent. Clophen A 60 had an average composition of 0.2% monochlorbiphenyl, 1.1% dichlorbiphenyl, 2.2% trichlorbiphenyl, 3.1% tetrachlorbiphenyl, 19.8% pentachlorbiphenyl, 43.2% hexachlorbiphenyl, 25.3% heptachlorbiphenyl, 4.7% octachlorbiphenyl, and 0.3% nonachlorbiphenyl; furans were absent (Wrabetz, 1979, personal communication).

During the experiment the animals were checked 5 days a week for death or morbidity. After Day 801, randomly selected animals from all three experimental groups were killed daily so that the experiment was completed after 832 days.

In general, necropsies were performed on all rats that had died or had been killed in a moribund condition. Rats which died but were not available for necropsy were designated "lost animals."

Tissues were fixed in Formalin and embedded in paraffin; 5-μm-thick sections were stained with hematoxylin and eosin (HE). Thymus, lungs, spleen, kidneys, ventral prostate, urethra with dorsal prostate, and urinary bladder were investigated routinely. Other tissues (e.g., skin, testes, pituitary gland, and glandula praeputialis) were examined when there was macroscopic indication of tumor or inflammation. The left lateral lobe of the liver was investigated in each case; other liver lobes were also taken when there was any indication of lesions or tumor-like changes. Liver lesions were classified into major types, consistent with those listed by Squire and Levitt (1975), by the Committee on Histologic Classification of Laboratory Animal Tumors (1980) and by Ward (1981). The significance of differences in incidence was tested with Fisher's exact test.

RESULTS

Mortality Rate

Table 1 shows the mortality rate for all three experimental groups in 100-day intervals, to day 800. Within the first 500 days of experiment, the mortality rate was relatively low. Between 501 and 600 days, the mortality rate in both the control and Clophen A 30 groups reached 12%, that in the Clophen A 60 group, 8%. In the next interval (601–700 days) mortality in the control group rose to 17%, whereas that in the Clophen A 30 group remained at 12%, and that in the Clophen A 60 group dropped to only 5%. In the last interval (Day 701–800) the mortality rate in controls was 42%, whereas that in group 2 was only 19% and that in group 3 was 35%. The total mortality in the first 800 days in the two treated groups (43 and 40%) was significantly lower than the mortality of 62% observed in the control group ($p < 0.05$).

Lesions Leading to Death up to Day 800 of the Experiment

By Day 800, 60% of the control animals, 37% of the Clophen A 30 animals, and 33% of the Clophen A 60 animals had been necropsied. The most frequent lesions leading to death or termination in these animals are shown in Table 2. The distribution of these lesions per 100-day interval is shown in Table 3 (a–e). Although the incidence of hepatocellular carcinoma was fairly low, differences were observed: in the first 800 days, liver carcinoma accounted for 21% of animals in the Clophen A 60 group but only 2% of animals in the Clophen A 30 group and none of control animals. The incidence of liver carcinomas in the two treated groups

TABLE 1

MORTALITY RATE IN CONTROL RATS (GROUP 1), RATS WITH CLOPHEN A 30-SUPPLEMENTED DIET (GROUP 2), AND RATS WITH CLOPHEN A 60-SUPPLEMENTED DIET (GROUP 3)

	Group 1 (N = 139)			Group 2 (N = 152)			Group 3 (N = 141)		
Interval (Days)	Dead or killed when moribund		Animals surviving at the beginning of time interval	Dead or killed when moribund		Animals surviving at the beginning of time interval	Dead or killed when moribund		Animals surviving at the beginning of time interval
101-200	(1)[a]	1%	139	0	0%	152	0	0%	141
201-300	(1)	1%	138	(2)	1%	152	(3)	2%	138
301-400	0	0%	137	3 (2)	2%	150	3 (2)	2%	135
401-500	11 (2)	8%	137	8 (2)	5%	147	4 (2)	3%	131
501-600	15 (2)	12%	126	17 (6)	12%	139	10 (5)	8%	121
601-700	19 (2)	17%	111	15 (2)	12%	122	6	5%	115
701-800	39	42%	92	20	19%	107	30	35%	85
1-800	86 (8)	62%	139	65 (14)[a]	43%	152	56 (12)[a]	40%	141

[a] Numbers in parentheses refer to animals dead but not available for necropsy.
[b] Significantly different ($p < 0.05$) compared to untreated group.

TABLE 2

MOST FREQUENTLY OCCURRING LESIONS FOUND IN NECROPSIED ANIMALS UNTIL DAY 800

	Group		
	1	2	3
N = number of animals at the start of the experiment minus animals not necropsied (lost animals)	131	138	129
n = number of animals necropsied until Day 800	78 (60%)	51 (37%)	44 (33%)
Neoplastic lesions			
Hepatocellular carcinoma	0	1	9[b]
% n	0	2	21
% N	0	1	7
Thymoma	16	4[b]	2[b]
% n	21	8	5
% N	12	3	4
Other neoplasias	52	28[b]	18[b]
% n	67	55	41
% N	40	20	14
Nonneoplastic lesions			
Urogenital system Prostatitis p. Abscess gl. praep.	19	15	7
% n	24	29	16
% N	15	11	5
Wistar-nephritis	9	0	0
% n	12	0	0
% N	7	0[b]	0[b]

[b] Significantly different ($p < 0.05$) compared to untreated group.

TABLE 3

RATE (%) OF THE MOST FREQUENTLY OCCURRING LESIONS LEADING TO DEATH, OBSERVED OVER INTERVALS OF 100 DAYS

Time interval (Days)	Group 1	Group 2	Group 3
	(a) Hepatocellular carcinoma		
301–400	0 (0/137)[a]	0 (0/150)	0 (0/135)
401–700	0 (0/111)	0 (0/122)	0 (0/115)
701–800	0 (0/92)	1 (1/107)	11* (9/85)
	(b) Thymoma		
301–400	0 (0/137)	0 (0/150)	0 (0/135)
401–500	1 (1/137)	0 (0/147)	1 (1/131)
501–600	4 (5/126)	1 (2/139)	0 (0/121)
601–700	5 (5/111)	0 (0/122)	1 (1/115)
701–800	5 (5/92)	2 (2/107)	0 (0/85)
	(c) Other neoplasias		
301–400	0 (0/137)	1 (1/150)	1 (2/135)
401–500	5 (7/137)	2 (3/147)	2 (2/131)
501–600	7 (9/126)	5 (7/139)	2 (3/121)
601–700	10 (11/111)	3 (4/122)	3 (4/115)
701–800	27 (25/92)	12* (13/107)	8* (7/85)
	(d) Urogenital system (prostatitis purulenta, pyelonephritis, abscess glandula praeputialis)		
301–400	0 (0/137)	1 (1/150)	0 (0/135)
401–500	3 (4/137)	2 (3/147)	0 (0/131)
501–600	3 (4/126)	2 (3/139)	1 (1/121)
601–700	5 (5/111)	3 (4/122)	1 (1/115)
701–800	7 (6/92)	5 (5/107)	6 (5/85)
	(e) Wistar-nephritis		
301–400	0 (0/137)	0 (0/150)	0 (0/135)
401–500	0 (0/137)	0 (0/147)	0 (0/131)
501–600	0 (0/126)	0 (0/139)	0 (0/121)
601–700	0 (0/111)	0 (0/122)	0 (0/115)
701–800	21 (9/92)	0* (0/107)	0* (0/85)

[a] Data are percentages and, in parentheses, proportions.
* Significantly different ($p < 0.05$) compared to untreated group.

up to Day 800 of experiment was 7 and 1%, respectively (Table 2). The tumors were first observed after 700 days. Thymoma accounted for 21% of animals in the control group (incidence 12%) but only 8% in the Clophen A 30 group (incidence 3%) and 5% in the Clophen A 60 group (incidence 4%). The thymoma rate in control animals remained constant between 500 and 800 days (Table 3b). The group of "other neoplasias" included tumors of the skin, testes, and pituitary gland. These tumors were also more frequent in the

control group (incidence 40%) than in either of the treated groups (incidence 20 and 14%), the rate rising steadily from Day 400 to 800 (Table 3c). Nonneoplastic lesions of the urogenital tract (i.e., prostatitis purulenta, abscess of glandula praeputialis, and Wistar-nephritis) accounted for 36% of necropsied control animals, 29% in the Clophen A 30 group, and 16% in the Clophen A 60 group. Prostatitis purulenta was observed in all groups with a higher incidence in the control group (15%) than in either of the treated groups (11 and 5%). The first cases were seen after Day 401 (Table 3d), the rate increasing slowly until Day 800. The severe form of Wistar-nephritis was only observed in control animals (incidence 7%).

Lesions in Animals Killed at the End of Experiment

Table 4 shows the frequency of major lesions in animals killed at the end of the experiment. Hepatocellular carcinoma was the most common lesion observed in the Clophen A 60 animals (61%), whereas it was only observed in 3% of animals in the Clophen A 30 group and 2% in the control group. In contrast, thymomas, observed in 17% of the control animals and 14% of the Clophen A 30 animals, were not identified in the animals treated with Clophen A 60. Other neoplasias were observed in 36% of the control animals and 38% of the Clophen A 30 animals but only 15% of the Clophen A 60 animals. After 800 days, nonneoplastic lesions of the urogenital tract, other than Wistar-nephritis, ceased to be of importance. The incidence of severe Wistar-nephritis rose to 40% in control animals. Even after 800 days, no cases of severe Wistar-nephritis were observed in the Clophen A 60 group, but the first cases were observed in the Clophen A 30 group (incidence 8%). Various pathological effects were observed in liver cells (e.g., hypertrophy of individual cells, hyperchromatic nuclei, foamy or vacuolated cytoplasm) but were not further investigated in this study. Similar findings have been described by Kimbrough *et al.* (1972) and are nonspecific effects associated with long-term feeding of Clophen.

TABLE 4

FREQUENCY OF MOST FREQUENTLY OCCURRING LESIONS IN ANIMALS KILLED AT THE END OF THE EXPERIMENT

Most frequent lesions	Group 1	2	3
Hepatocellular carcinoma	2 (1/53)[a]	3 (3/87)	61[b] (52/85)
Thymoma	17 (9/53)	14 (12/87)	0[b] (0/85)
Other neoplasias	36 (19/53)	38 (33/87)	15 (13/85)
Urogenital system	0 (0/53)	1 (1/87)	0 (0/85)
Wistar-nephritis	40 (21/53)	8[b] (7/87)	0 (0/85)

[a] Data are percentages and, in parentheses, proportions.
[b] Significantly different ($p < 0.05$) compared to untreated animals.

Preneoplastic lesions such as foci of hepatocellular alterations (Fig. 1) and neoplastic nodules (Fig. 2) were regularly observed. Their incidence is shown in Table 5 together with the rate of hepatocellular carcinoma. Only the highest stage of neoplastic development (i.e., focus of hepatocellular alterations or neoplastic nodule or hepatocellular carcinoma) per animal is included. In general foci and nodules were first observed after Day 500. The incidence in control animals was low until Day 800 after which the incidence of foci rose to 32%, the number of neoplastic nodules and neoplastic lesions remaining low (4 and 2%, respectively). The incidence of preneoplastic and neoplastic lesions in necropsied Clophen A 30 animals remained constant at 50–60% between Day 500 and 800, rising to 100% in animals killed

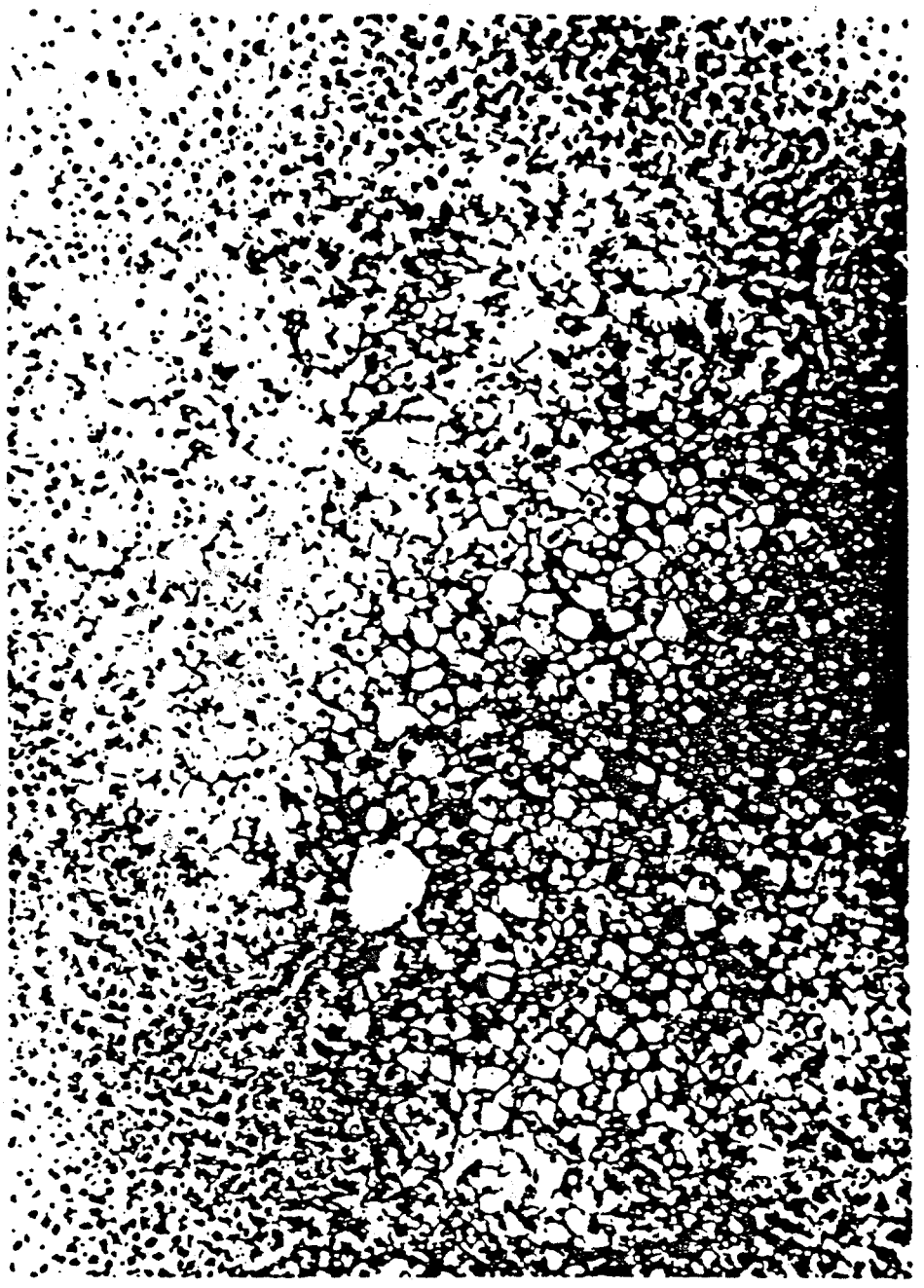

FIG. 1. Clear cell area of altered hepatocytes in a rat fed Clophen A 60. Note empty appearance of cytoplasm of altered cells. HE × 140.

after Day 800. The incidence of foci predominated in all time intervals, but an increase in neoplastic nodules and hepatocellular carcinoma (Fig. 3) was observed with increased time. Preneoplastic or neoplastic lesions were observed in 100% of the Clophen A 60

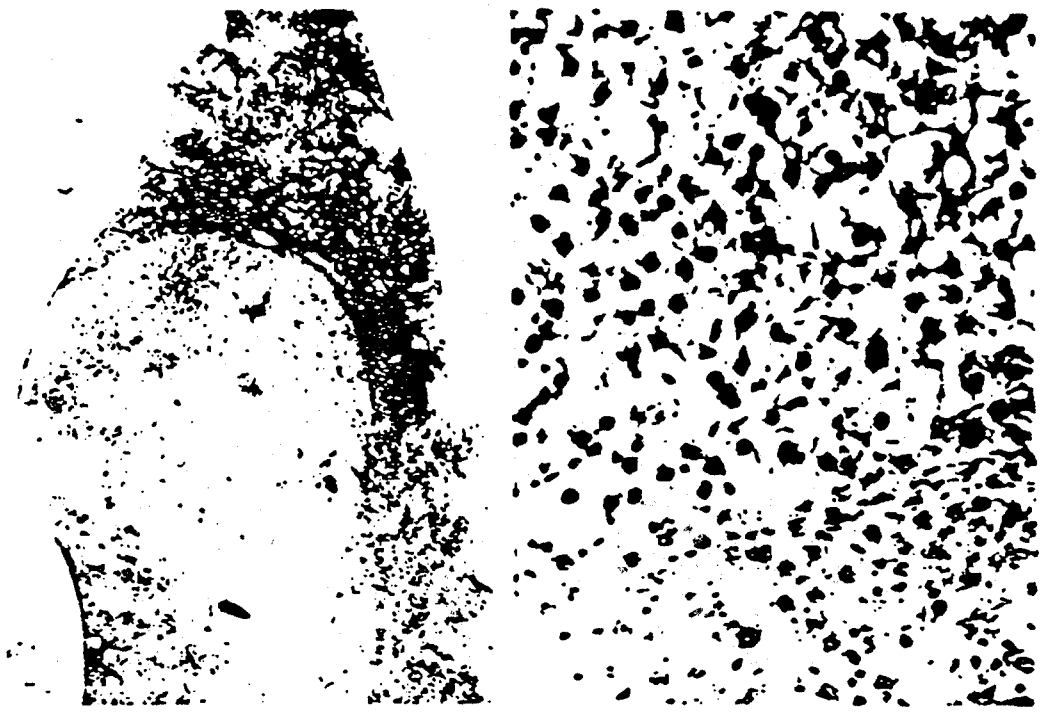

FIG. 2. Left side. Neoplastic nodule in a rat fed Clophen A 30. Periphery is sharply demarcated from surrounding normal liver parenchyma. HE × 13. Right side. Edge of the same neoplastic nodule. The surrounding liver plates are compressed. HE × 323.

animals necropsied after Day 500. There was a time-dependent trend from foci to neoplastic nodules to hepatocellular carcinoma.

Nonneoplastic proliferative lesions i.e., bile duct hyperplasia and associated lesions such as adenofibrosis and cysts (cholangiomas),

TABLE 5

FREQUENCY OF NECROPSIED ANIMALS WITH FOCI OF HEPATOCELLULAR ALTERATIONS, NEOPLASTIC NODULES, AND HEPATOCELLULAR CARCINOMAS (ONLY THE HIGHEST STAGE OF LIVER CARCINOGENESIS PER ANIMAL IS INCLUDED)

	Group								
	1			2			3		
Time interval (Days)	Foci	Neoplastic nodules	Hepatocellular carcinoma	Foci	Neoplastic nodules	Hepatocellular carcinoma	Foci	Neoplastic nodules	Hepatocellular carcinoma
301–400	0 (0/0)[a]	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/1)	100 (1/1)	0 (0/1)
401–500	11 (1/9)	0 (0/9)	0 (0/9)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)
501–600	8 (1/13)	8 (1/13)	0 (0/13)	55 (6/11)	0 (0/11)	0 (0/11)	40 (2/5)	60 (3/5)	0 (0/5)
601–700	6 (1/17)	12 (2/17)	0 (0/17)	33 (4/12)	17 (2/12)	0 (0/12)	0 (0/5)	100 (5/5)	0 (0/5)
701–800	5 (2/39)	0 (0/39)	0 (0/39)	50 (10/20)[b]	5 (1/20)	5 (1/20)	3 (1/30)	67 (20/30)[b]	30 (9/30)[b]
801–832	32 (17/53)	4 (2/53)	2 (1/53)	49 (43/87)	40 (35/87)[b]	3 (3/87)	0 (0/85)[b]	40 (34/85)[b]	61 (52/85)[b]

[a] Data are percentages and, in parentheses, proportions.
[b] Significantly different ($p < 0.05$) compared to untreated group.

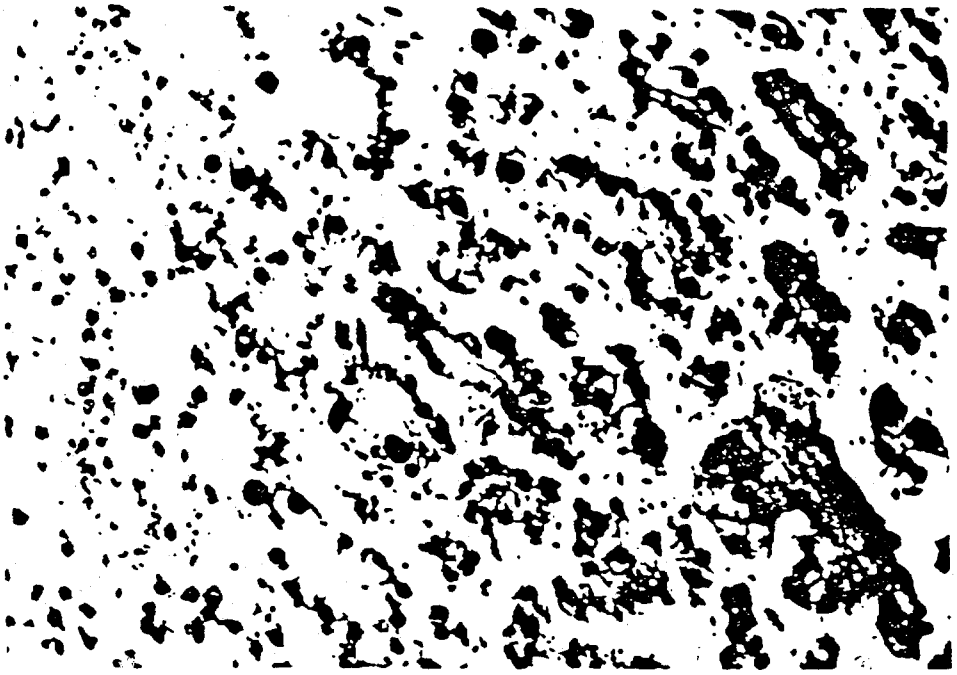

FIG. 3 Hepatocellular (trabecular) carcinoma in a rat fed Clophen A 60. Neoplastic cells are arranged in trabecular cords and irregular nests; note hepatocellular pleomorphism. HE × 243.

were seen in both control and treated groups. The incidences of such lesions are shown in Table 6. However, they were not further studied because there is no convincing evidence that they are precancerous lesions (Stewart and Snell, 1957).

DISCUSSION

Clophen A 60 had a definite, and Clophen A 30 a weak, carcinogenic effect on rat liver in these experiments. The first hepatocellular carcinomas were detected after a latency

TABLE 6

NONNEOPLASTIC PROLIFERATIVE LIVER LESIONS

Time interval (Days)	Group 1			Group 2			Group 3		
	Bile duct hyperplasia	Adeno-fibrosis	Cysts	Bile duct hyperplasia	Adeno-fibrosis	Cysts	Bile duct hyperplasia	Adeno-fibrosis	Cysts
401–500	50 (5/9)[a]	0 (0/9)	0 (0/9)	0 (0/6)	0 (0/6)	0 (0/6)	50 (1/2)	0 (0/2)	0 (0/2)
501–600	38 (5/13)	0 (0/13)	0 (0/13)	27 (3/11)	0 (0/11)	0 (0/11)	60 (3/5)	0 (0/5)	0 (0/5)
601–700	29 (5/17)	0 (0/17)	0 (0/17)	25 (3/12)	0 (0/12)	0 (0/12)	83 (5/6)	0 (0/6)	0 (0/6)
701–800	31 (12/39)	18 (7/39)	0 (0/39)	48 (10/21)	5 (1/21)	0 (0/21)	73 (22/30)[b]	3 (1/30)	3 (1/30)
801–832	13 (7/53)	23 (12/53)	0 (0/53)	32 (28/87)[b]	7 (6/87)[b]	0 (0/87)	73 (62/85)[b]	2 (2/85)[b]	12 (10/85)[b]

[a] Data are percentages and, in parentheses, proportions.

[b] Significantly different ($p < 0.05$) compared to untreated group.

period of 700 days in both groups, but with a higher incidence in the Clophen A 60 group. The incidence of hepatocellular carcinoma in the Clophen A 60 group reached 61% at the end of the experiment. Although only 3% of the Clophen A 30 group showed hepatocellular carcinomas after 800 days, 89% of the animals had preneoplastic lesions. These findings confirm the observations of Ito *et al.* (1974) that hepatocellular carcinomas emerge in rats after administration of PCBs (Kanechlor) for more than 364 days. The findings are also consistent with the results described by McConnell (1980), i.e., that the occurrence of neoplastic nodules in rats (Ito *et al.*, 1974) and mice (Nagasaki *et al.*, 1972) following administration of Japanese commercial mixtures of PCBs is related to the number and location of the halogen atoms by which the mixtures are classified. Both the DHEW Subcommittee on Health Effects of PCBs and PBBs (1978) and Ecobichon (1975) have reported that the toxic potency of PCBs (hepatic enzyme induction, hepatocarcinogenic effect) increases with increasing chlorination and chlorine substitution in the *para, ortho, meta* positions, respectively.

The high incidence of preneoplastic and neoplastic lesions in the livers indicates a carcinogenic effect of both PCB mixtures. The application of the higher chlorinated Clophen A 60 results in an appreciable number of hepatocellular carcinomas, whereas with the lower chlorinated Clophen A 30 numerous preneoplastic foci were found. According to the present knowledge, PCBs are regarded to be promoters rather than initiators. However, it should be mentioned that long-term feeding of potential carcinogenic compounds as in the present experiment may not allow distinction between the initiating and the promoting activity of a chemical substance (Schulte Hermann *et al.*, 1983).

The maximum incidence of neoplastic nodules was observed in the Clophen A 60 group in the period Day 601 to 700 (after which the increase in hepatocellular carcinoma accounted for the drop in neoplastic nodules) and in the Clophen A 30 group at the end of the experiment. The incidence of neoplastic nodules in untreated animals was very low. This is consistent with the observations that neoplastic nodules are rare in untreated rats, except in aged rats, and that they are only induced by hepatocarcinogens (Farber, 1963; Marugami *et al.*, 1967; Ito *et al.*, 1969, 1974; Kimbrough, 1979; Pollard and Luckert, 1979; Committee on Histologic Classification of Laboratory Animal Tumors, 1980).

Foci of cellular alterations were observed in all three groups after Day 500, but again the greatest occurrence was observed in treated animals. Although foci are known to appear in old untreated rats, increased numbers encountered in bioassay studies are accepted as an indication of possible carcinogenicity (Committee on Histologic Classification of Laboratory Animal Tumors, 1980). In the rat, foci have also been shown to represent stages in the development of hepatocellular carcinoma (Gössner and Friedrich-Freksa, 1964; Bannasch, 1968; Fischer *et al.*, 1983). The promoting activity of PCBs on preneoplastic enzyme-altered foci and islands in rat liver has been described recently by Pereira *et al.* (1982) and by Oesterle and Deml (1983).

The total mortality rate in the experiment was significantly reduced by Clophen administration. This mainly resulted from the marked appearance of thymoma and other (nonliver) neoplasias, in purulent inflammatory lesions of the urogenital system, and of Wistar-nephritis commonly found in old rats (Bullock *et al.*, 1968) (see Tables 2 and 3b–e). This may be explained by an interaction with the immune system. In recent years a number of compounds have been shown to alter immunocompetence (Vos *et al.*, 1980; Chang *et al.*, 1981; Fraker, 1980; IARC, 1978). In particular, Vos *et al.* (1980) have suggested that PCBs may modulate host defense mechanisms and immune responses. Among other lesions, atrophy of lymphoid

tissues, such as spleen, lymph nodes, and thymus, has been induced by treatment with PCBs in chickens, rabbits, and guinea pigs (Vos *et al.*, 1980), and by polybrominated biphenyls in mice (Fraker, 1980). Moreover macrophage functions, as determined by *in vivo* bacterial clearance, are inhibited by PCB exposure (Vos *et al.*, 1980). Wistar-nephritis is thought to be immunopathic (Fridman-Manduzio *et al.*, 1980; Hirokawa, 1975). Thus the protection from neoplasms of the thymus and from Wistar-nephritis in the Clophen-treated groups in the present experiment might result from PCB-induced alterations in the immune system.

Furthermore the present study supports the conclusion of the DHEW Subcommittee on Health Effects of PCBs and PBBs that the liver is the primary target organ for PCBs in the rat.

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During the PWG review, the PWG panel examined all sections of liver in which a diagnosis of a hepatocellular neoplasm or nodular hyperplasia had been either diagnosed by the reviewing pathologist or reported in the NCI Technical Report.

No hepatocellular neoplasms were observed in control male or female rats. Hepatocellular adenomas and/or carcinomas were present in low numbers in all treated groups. When present, they occurred as singular tumors and none of the affected rats had both a hepatocellular adenoma and a hepatocellular carcinoma. The incidences of hepatocellular neoplasms present in male and female F344 rats are summarized as follows:

Male F344 Rats

	Control	Low	Mid	High
No. Examined	24	24	24	23
Hepatocellular Adenoma	0 (0%)	1 (4.2%)	1 (4.2%)	1 (4.3%)
Hepatocellular Carcinoma	0 (0%)	0 (0%)	0 (0%)	2 (8.7%)
Total Animals with Hepatocellular Neoplasms	0 (0%)	1 (4.2%)	1 (4.2%)	3 (13%)

Female F344 Rats

	Control	Low	Mid	High
No. Examined	23	24	24	24
Hepatocellular Adenoma	0 (0%)	1 (4.2%)	2 (8.3%)	1 (4.2%)
Hepatocellular Carcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total Animals with Hepatocellular Neoplasms	0 (0%)	1 (4.2%)	2 (8.3%)	1 (4.2%)

Most of the hepatocellular lesions which were reported as hyperplasia, nodular in the NCI Technical Report were considered to be foci of cellular alteration when reexamined by the PWG. An increased number of foci of cellular alteration were present in treated groups in this study and consisted primarily of basophilic, eosinophilic and mixed cell foci. The incidences of foci of cellular alteration in the liver are summarized as follows:

Male F344 Rats

	Control	Low	Mid	High
No. Examined	24	24	24	23
Focus/Foci, Eosinophilic	0 (0%)	4 (16.7%)	5 (20.8%)	4 (17.4%)
Focus/Foci, Basophilic	0 (0%)	1 (4.2%)	0 (0%)	7 (30.4%)
Focus/Foci, Mixed Cell	0 (0%)	2 (8.3%)	4 (16.7%)	8 (34.8%)
Focus/Foci, Clear Cell	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total Animals with Any Type of Focus/Foci	0 (0%)	7 (29.2%)	9 (37.5%)	16 (69.6%)

Female F344 Rats

	Control	Low	Mid	High
No. Examined	23	24	24	24
Focus/Foci, Eosinophilic	0 (0%)	10 (41.7%)	13 (54.2%)	6 (25%)
Focus/Foci, Basophilic	2 (8.7%)	2 (8.3%)	0 (0%)	4 (16.7%)
Focus/Foci, Mixed Cell	0 (0%)	1 (4.2%)	0 (0%)	5 (20.8%)
Focus/Foci, Clear Cell	0 (0%)	0 (0%)	1 (4.2%)	2 (8.3%)
Total Animals with Any Type of Focus/Foci	2 (8.7%)	12 (50%)	14 (58.3%)	15 (62.5%)

Other lesions which occurred more frequently in Aroclor 1254 treated rats included centrilobular hepatocytomegaly which occurred in a few treated rats of each sex in each group and pigment deposition which occurred frequently in treated females, often in conjunction with focal/multifocal granulomatous hepatitis. The incidence of these nonneoplastic changes are presented as follows:

Male F344 Rats

	Control	Low	Mid	High
No. Examined	24	24	24	23
Centrilobular Hepatocytomegaly	0 (0%)	3 (12.5%)	3 (12.5%)	3 (13%)
Pigment Deposition	0 (0%)	0 (0%)	2 (8.3%)	2 (8.7%)
Focal/Multifocal Granulomatous Hepatitis	0 (0%)	0 (0%)	1 (4.2%)	3 (13%)

	Female F344 Rats			
	Control	Low	Mid	High
No. Examined	23	24	24	24
Centrilobular Hepatocytomegaly	0 (0%)	3 (12.5%)	1 (4.2%)	3 (12.5%)
Pigment Deposition	3 (13%)	16 (66.7%)	16 (66.7%)	18 (75%)
Focal/Multifocal Granulomatous Hepatitis	3 (13%)	4 (16.7%)	11 (45.8%)	14 (58.3%)

SUMMARY AND CONCLUSIONS

Of the six studies reviewed by the PWG, four studies used the PCB Aroclor 1260 or its equivalent, Clophen A 60. Three of these studies were long-term studies to examine the potential chronic toxicity and carcinogenic activity of Aroclor 1260 or Clophen A 60. The fourth study was a reproduction study in which the F_0 generation male and female rats were examined following nine months of exposure to 100 ppm Aroclor 1260. The other two studies examined by the PWG included long-term studies of Clophen A 30 and Aroclor 1254. These chemicals differ from Aroclor 1260 and Clophen A 60, in that they both have a lower chlorine content. Clophen A 30 has the lowest chlorine content of the chemicals reviewed.

The studies reviewed varied in the strain of rat used, the sex of rat utilized, and the age of sacrifice for tissue examination. The results of the PWG review revealed that the most prominent

difference determining the carcinogenic potential was the specific chemical tested. Additionally, strain and sex differences were noted in the spectrum of liver lesions present in the various studies reviewed.

In F_0 generation male and female Sherman rats that received 100 ppm of Aroclor 1260 for nine months in a reproduction study, centrilobular hepatocytomegaly was the principle effect and appeared to be slightly more severe in male rats than in female rats at this age. However, the early appearance of small eosinophilic foci occurred in female Sherman rats as did the deposition of brown pigment in Kupffer cells. The overall degree of toxicity in the liver of male and female rats following nine months of treatment was judged to be minimal and the centrilobular hepatocytomegaly represents an adaptive hepatocellular response to Aroclor 1260 by the liver.

One of the objectives of the PWG review was to compare the results of the long-term studies which were conducted in various strains of rats with different polychlorinated biphenyls. The incidence of hepatic neoplasms, foci of cellular alteration and centrilobular hepatocytomegaly are presented for each of the long-term studies on the following page:

Comparison of PWG Results for Chronic Studies Conducted in
Four Strains of Rats with Aroclor 1260, Clophen a 60, Clophen a 30 and Aroclor 1254*

Chemical:	Aroclor 1260		Aroclor 1260				Clophen			Aroclor 1254			
Strain:	Sherman		Sprague-Dawley				Wistar			Fischer 344			
Dose:	100 ppm		100 ppm				100 ppm			100 ppm			
Sex:	Female		Male		Female		Male			Male		Female	
	Control	Test	Control	Test	Control	Test	Control	A 60	A 30	Control	Test	Control	Test
No. Examined	187	189	31	40	45	46	120	125	128	24	23	23	24
Hepatocellular Adenoma	0	135	0	4	1	29	6	85	14	0	1	0	1
Hepatocellular Carcinoma	1	21	0	1	0	19	2	67	2	0	2	0	0
Animals with Hepatocellular Adenoma and/or Carcinoma	1	138	0	5	1	41	8	114	16	0	3	0	1
Cholangiocarcinoma	0	0	0	0	0	3	1	0	0	0	0	0	0
Centrilobular Hepatocytomegaly	1	108	0	15	0	5	1	2	2	0	3	0	3
Focus/Foci, Eosinophilic	7	173	1	16	5	36	51	101	98	0	4	0	6
Focus/Foci, Basophilic	4	67	1	0	2	1	2	4	15	0	7	2	4
Focus/Foci, Clear Cell	14	67	4	0	1	0	7	28	39	0	0	0	2
Focus/Foci, Mixed Cell	1	38	0	2	0	0	7	28	49	0	8	0	5
Animals with Focus/Foci of Any Type	25	177	5	16	7	36	55	108	106	0	16	2	15

*Aroclor 1254 given at dosages of 25, 50 and 100 ppm. Only data for 100 ppm groups presented for comparative purposes.

In female Sherman rats that received 100 ppm of Aroclor 1260 for up to 23 months, 73% of the treated rats examined were diagnosed with hepatocellular neoplasms. The overwhelming majority of these neoplasms were benign (62% of the treated rats examined had only hepatocellular adenomas; approximately 11% of the rats had hepatocellular carcinomas or both benign and malignant tumors). Three rats had unusual tumors with glandular, papillary patterns giving the appearance of both hepatocellular epithelium and biliary epithelium simultaneously within a single tumor. Multiple eosinophilic foci occurred in nearly all of the treated rats examined, frequently in rats already diagnosed with hepatocellular neoplasms. The distinction between large eosinophilic foci with enlarged hepatocytes exhibiting compression and hepatocellular adenomas was sometimes very difficult. Other types of foci were also increased in treated rats. Centrilobular hepatocytomegaly was also a very frequent finding occurring in over 50% of the treated rats.

In male and female Sprague-Dawley rats that received Aroclor 1260 at 100 ppm for 16 months, followed by eight months at 50 ppm and up to an additional five months on laboratory chow, 12.5% of the males and approximately 90% of the females had hepatocellular neoplasms. In males, four of the five neoplasms were diagnosed as hepatocellular adenomas. In females, approximately equal numbers of benign and malignant neoplasms were diagnosed with approximately 48% having only

benign neoplasms and approximately 41% of the female rats having malignant or both benign and malignant neoplasms. However, it was noted that a few of the rats in this study were subjected to surgical procedures (partial hepatectomy). The time on study for the rats examined varied as much as 12 months with tissues not examined from rats prior to 18 months, then examined at monthly intervals thereafter up to 29 months. These factors could affect the incidence of various neoplasms.

Hepatocellular tumors appearing to have mixed hepatocellular and biliary elements occurred in four female rats and biliary neoplasms (cholangiocarcinoma) occurred in three treated female rats. These neoplasms usually occurred in livers in which purely hepatocellular neoplasms had already been diagnosed. Eosinophilic foci occurred in slightly less than half of the treated males and in more than three-fourths of the treated females. These lesions were similar to those noted in the female Sherman rats and occasionally the distinction between large eosinophilic foci with enlarged hepatocytes exhibiting compression and hepatocellular adenomas was sometimes very difficult. Centrilobular hepatocytomegaly was a common diagnosis in treated males similar to that noted in male Sherman rats sacrificed at nine months. In females, only a few rats were diagnosed with this lesion, but this may be due to the limited amount of nonneoplastic tissue available for examination.

In male Wistar rats that received 100 ppm of Clophen 60 for up to 832 days, approximately 91% of the rats examined were diagnosed with hepatocellular neoplasms. Approximately 38% of the rats had benign neoplasms only, while 54% of the rats had hepatocellular carcinomas or both benign and malignant neoplasms. This is a reversal of the proportions noted in the study utilizing female Sherman rats in which the majority of the rats had only benign neoplasms. However, it should be noted that the length of the time the rats received the test material was also increased. Tumors appearing to have mixed hepatocellular and biliary elements occurred in nine of the treated rats, frequently in livers in which purely hepatocellular tumors also had been diagnosed. Eosinophilic foci occurred in approximately 80% of the treated rats examined. However, the eosinophilic foci in this study were smaller and blended better with adjacent parenchyma compared to the large compressive foci in the study with female Sherman rats. Centrilobular hepatocytomegaly was not a frequent finding in the treated male Wistar rats but the diagnosis of this lesion may have been hampered by the limited amount of hepatocellular tissue available for examination from each rat. Frequently, only single sections of liver lobes were available and the entire section was neoplastic.

The principle toxic change noted in rats receiving Aroclor 1260 or Clophen A 60 appeared to be proliferative in nature. Beginning with centrilobular hepatocytomegaly, probably due to enzyme induction

resulting in proliferation of smooth endoplasmic reticulum and cell hypertrophy, the affected livers appear to progress to foci of cellular alteration (principally eosinophilic) and ultimately to neoplasia. Hepatocellular hyperplasia was only infrequently diagnosed in the rats treated with Aroclor 1260 due to the overall absence of significant areas of degeneration or necrosis which would have triggered attempted regeneration of hepatic parenchyma. Although bile duct proliferation, oval cell proliferation and/or periportal fibrosis were present as treatment-related lesions in some of the studies, these lesions were not usually very severe, and there did not appear to be any significant loss of hepatic parenchyma in these rats.

In male Wistar rats that received 100 ppm of Clophen A 30 for up to 832 days the incidence of hepatocellular carcinomas was identical in the control and test group. A mild increase in the incidence of hepatocellular adenoma was present in the treated group as compared to the control group (5% in the control and 11% in the treated group). Increased incidences of foci of cellular alteration of hepatocytes was present in male Wistar rats given 100 ppm Clophen A 30. As noted for Wistar rats given Clophen A 60, centrilobular hepatocytomegaly was not a frequent finding but this observation may have been affected by the tissue sampling limiting the amount of hepatic parenchyma available for microscopic examination.

Administration of Aroclor 1254 to male and female Fischer 344 rats at dosages of 25, 50 and 100 ppm for 104-105 weeks resulted in an increase in the incidence of foci of cellular alteration in the liver. A few hepatocellular neoplasms were diagnosed in treated rats, however; the total number of affected rats was small and within the expected range for rats of this age and strain. Centrilobular hepatocytomegaly and pigment deposition, often occurring with multifocal granulomatous hepatitis, also occurred more frequently in Aroclor 1254 treated rats.

Jerry F. Hardisty D.V.M.

JERRY F. HARDISTY, D.V.M.
PWG Chairman

June 27, 1991
Date

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APPENDIX A

Aroclor 1260 in Female Sherman Rats
(Kimbrough, et al., 1975)

Group A1 - Control
Group A2 - 100 ppm Aroclor 1260

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

SUMMARY INCIDENCE TABLES

800705

SUMMARY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

	GROUP A1	GROUP A2				
LIVER, CONSENSUS (NO. EXAMINED)	(187)	(189)				
Hepatocellular Adenoma		135				
Hepatocellular Carcinoma	1	21				
Leukemia, Granulocytic	1					
Leukemia, Lymphocytic	1					
Lymphoma, Malignant	1					
Angiectasis	1	17				
Bile Duct Hyperplasia	4	29				
Cellular Atypia, Diffuse		1				
Cholangiofibrosis		3				
Cystic Bile Duct	1	2				
Degeneration, Cystic	2	7				
Dilated Bile Ducts, Focal		14				
Extramedullary Hematopoiesis	1					
Fatty Change, Centrilobular	1	32				
Fatty Change, Diffuse	1	6				
Fatty Change, Focal	2	4				
Focus/Foci, Basophilic	4	67				
Focus/Foci, Clear Cell	14	67				
Focus/Foci, Eosinophilic	7	173				
Focus/Foci, Mixed Cell	1	38				
Hepatitis, Granulomatous,						
Multifocal		1				
Hepatitis, Necrotizing,						
Multifocal	1	1				
Hepatitis, Suppurative,						
Multifocal	1	2				
Hepatocytomegaly,						
Centrilobular	1	108				
Hepatocytomegaly, Diffuse		3				
Hepatocytomegaly, Periportal		1				
Leukocytosis	1					
Necrosis, Centrilobular	1					
Necrosis, Focal		13				
Necrosis, Multifocal		1				
Oval Cell Proliferation	2	17				
Pigment Deposition	9	66				

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HISTOPATHOLOGY INCIDENCE TABLES

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HISTOPATHOLOGY INCIDENCE TABLE

GROUP A1

Liver Reassessment
CDC/1260 Sacrifice
Female Rat

ANIMAL	81119	81120	81121	81122	81123	81124	81125	81126	81127	81128	81129	81130	81131	81132	81133	81134	81135	81136	81137	81138
LIVER, CONSENSUS	X	X	X		X	X	N	X	X	X		X	X	X	X	X	X	X		X
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia																				
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell											1								1	
Focus/Foci, Eosinophilic																				
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pigment Deposition				2																

EPL Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A1

ANIMAL	81391	81402	81412	81423	81434	81444	81455	81466	81477	81488	81499	81501	81511	81522	81533	81544	81555	81566	81577	81588
LIVER, CONSENSUS	X		X	X	X	N	X	X	N	X			X	X	X	X	X	X	X	X
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia																				
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell												1								
Focus/Foci, Eosinophilic																				
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal											1									
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation		2																		
Pigment Deposition		3																		

EPL Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A1

ANIMAL	8159	8160	8161	8162	8163	8164	8165	8166	8167	8168	8169	8170	8171	8172	8173	8174	8175	8176
LIVER, CONSENSUS	X	X			X	X	X	X	X	X	X	X		X		X		X
Hepatocellular Adenoma																		
Hepatocellular Carcinoma																		
Leukemia, Granulocytic																		
Leukemia, Lymphocytic																		
Lymphoma, Malignant																		
Angiectasis																		
Bile Duct Hyperplasia																		
Cellular Atypia, Diffuse																		
Cholangiofibrosis																		
Cystic Bile Duct																	3	
Degeneration, Cystic															1			
Dilated Bile Ducts, Focal																		
Extramedullary Hematopoiesis																		
Fatty Change, Centrilobular													3					
Fatty Change, Diffuse			5															
Fatty Change, Focal																		
Focus/Foci, Basophilic																		
Focus/Foci, Clear Cell				1														
Focus/Foci, Eosinophilic																		
Focus/Foci, Mixed Cell																		
Hepatitis, Granulomatous, Multifocal																		
Hepatitis, Necrotizing, Multifocal																		
Hepatitis, Suppurative, Multifocal																		
Hepatocytomegaly, Centrilobular																		
Hepatocytomegaly, Diffuse																		
Hepatocytomegaly, Periportal																		
Leukocytosis																		
Necrosis, Centrilobular																		
Necrosis, Focal																		
Necrosis, Multifocal																		
Oval Cell Proliferation																		
Pigment Deposition																		

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=s
P=Present B=Benign M=Malignant
m=missing one paired organ u=[illegible]

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

	GROUP A1																			
ANIMAL	81179	81180	81181	81182	81183	81184	81185	81186	81187	81188	81189	81190	81191	81192	81193	81194	81195	81196	81197	81198
LIVER, CONSENSUS	X	X	X	X	N	X			X		X	X	X		X	X	X	X	X	X
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia							1													
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic										2										
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell								1		2				3						
Focus/Foci, Eosinophilic										2										
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pigment Deposition																				

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A1

ANIMAL	81999	82000	82001	82002	82003	82004	82005	82006	82007	82008	82009	82010	82011	82012	82013	82014	82015	82016	82017	82018
LIVER, CONSENSUS		X	X	X	X	X		X	X	X		X	X	X		X	X	N	X	X
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant							P													
Angiectasis																				
Bile Duct Hyperplasia																				
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic	1																			
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic											2				1					
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pigment Deposition																				

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

	GROUP A1																			
ANIMAL	821 9	822 0	822 1	822 2	822 3	822 4	822 5	822 6	822 7	822 8	822 9	823 0	823 1	823 2	823 3	823 4	823 5	823 6	823 7	823 8
LIVER, CONSENSUS				X			X	X			X					X	X	X		X
Hepatocellular Adenoma													P							
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis					1															
Bile Duct Hyperplasia													3							
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal												2								
Focus/Foci, Basophilic		3																		
Focus/Foci, Clear Cell			1						2					2	1				2	
Focus/Foci, Eosinophilic																				
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pigment Deposition	1					1				2			2							

EPL
Experimental Pathology Laboratories, Inc.

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A1

ANIMAL	823 9	824 0	824 1	824 2	824 3	824 4	824 5	824 6	824 7	824 8	824 9	825 0	825 1	825 2	825 3	825 4	825 5	825 6	825 7	825 8
LIVER, CONSENSUS	X	X	X	X	X	X	X	X		X	X	X	X	X		X		N	X	X
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia																	1			
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
ıtty Change, Centrilobular																				
ratty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic																				
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis															4					
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation															3					
Pigment Deposition									3											

EPL | Experimental Pathology Laboratories, Inc.

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

	GROUP A1																			
ANIMAL	82591	82601	82611	82622	82633	82644	82655	82666	82677	82688	82699	82701	82712	82723	82734	82745	82756	82767	82778
LIVER, CONSENSUS		X		N	X	X	X	X	X	X	X	X		X	X	N		N	X	X
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																P				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia																				
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic	2											4								
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal			2																	
Hepatocytomegaly, Centrilobular												3								
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pigment Deposition																				

EPL | Experimental Pathology Laboratories, Inc.

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m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A1

ANIMAL	82791	82801	82811	82822	82833	82844	82855	82866	82877	82888	82899	82900	82911	82922	82933	82944	82955	82966	82977	82988
LIVER, CONSENSUS	X	X		X	X	X	X	X	N	X		X	X	X		X			N	N
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia																				
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis															3					
tty Change, Centrilobular																				
atty Change, Diffuse																				
Fatty Change, Focal											1									
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																	1			
Focus/Foci, Eosinophilic			2																	
Focus/Foci, Mixed Cell															2					
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pigment Deposition															1			1		

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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P=Present B=Benign M=Malignant
m=missing one paired organ u=[illegible] sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A1

ANIMAL	8299	8300	8301	8302	8303	8304	8305	8306	8307	8308	8309	8310	8311	8312	8313	8314	8315	8316	8317	8318
LIVER, CONSENSUS	X	X		N		X		X	X	X	X	X			X	X	X	X	X	X
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic							P													
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia			1																	
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic														1						
Focus/Foci, Clear Cell					2															
Focus/Foci, Eosinophilic													3							
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular							5													
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pigment Deposition																				

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	83119	83202	83212	83222	83232	83242	83252	83262	83272	83282	83292	83303	83313	83323	83333	83343	83353	83363	83373	83383
LIVER, CONSENSUS		X		N	N							N								
Hepatocellular Adenoma	P		P				P	P	P	P	P			P		P	P			P
Hepatocellular Carcinoma													P							
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis									3											
Bile Duct Hyperplasia									2		3				1					
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic														2						3
Dilated Bile Ducts, Focal								2												
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular	1								1											
Fatty Change, Diffuse																1				
Fatty Change, Focal																				
Focus/Foci, Basophilic			5			3	5		5							3	3		3	
Focus/Foci, Clear Cell	2					3	3		2					3	3					
Focus/Foci, Eosinophilic	4		5			5	4	5	5	4	4		4	5	5	4	5	1	5	3
Focus/Foci, Mixed Cell											3		2		3				2	
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal															2					
Hepatocytomegaly, Centrilobular	2						4	3	2	4				2	4		3	2	3	
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation			2										2				1			
Pigment Deposition			3						2	2	1		2	2	2	2	2			

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	8339	8340	8341	8342	8343	8344	8345	8346	8347	8348	8349	8350	8351	8352	8353	8354	8355	8356	8357	8358
LIVER, CONSENSUS					•					N	N									
Hepatocellular Adenoma	P	P	P	P			P	P				P			P		P			
Hepatocellular Carcinoma	P											P								
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia	2	3							3											
Cellular Atypia, Diffuse									P											
Cholangiofibrosis		3																		
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal		2										1								
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular						2														
Fatty Change, Diffuse																				
Fatty Change, Focal					1															
Focus/Foci, Basophilic			2		2	3		3										4		
Focus/Foci, Clear Cell			3					2							3					3
Focus/Foci, Eosinophilic	5	5	5	5	5	4	5	5				3	4	3	4	3	2	4	2	4
Focus/Foci, Mixed Cell			2										3							
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular				3		3						2	3			2		4		
Hepatocytomegaly, Diffuse									4										2	
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal					1		2													
Necrosis, Multifocal		3																		
Oval Cell Proliferation		2							3											
Pigment Deposition	2							2	3									3		

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	83591	83602	83613	83626	83634	83645	83656	83667	83678	83689	83701	83712	83723	83734	83745	83756	83767	83778	83789
LIVER, CONSENSUS																			
Hepatocellular Adenoma		P	P		P		P	P	P	P	P				P	P	P		P	P
Hepatocellular Carcinoma																			P	
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																			3	
Bile Duct Hyperplasia									2					2					4	
Cellular Atypia, Diffuse																				
Cholangiofibrosis														4						
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal										2										
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular		1				1		1	1	1			1	1						
Fatty Change, Diffuse																				
Fatty Change, Focal					1															
Focus/Foci, Basophilic			3				2	3	2		2			3	2		3			1
Focus/Foci, Clear Cell			2	1			2						2		3	2				2
Focus/Foci, Eosinophilic	3	4	5	5	4	3	3	5	5	3	4	3	5	2	5	5	5	3	5	4
Focus/Foci, Mixed Cell		4						4						3		4		4		
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular	3	2		3	3	1		1	2		3	1	2	1		3		3		2
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																			2	
Necrosis, Multifocal																				
Oval Cell Proliferation															3				3	
Pigment Deposition			2	2						2					2	1			2	

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	83709	83801	83812	83823	83834	83845	83856	83867	83889	83890	83901	83912	83923	83934	83945	83956	83967	83978	83989
LIVER, CONSENSUS	N																		
Hepatocellular Adenoma			P		P			P		P		P		P	P		P	P	P	
Hepatocellular Carcinoma								P							P					
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																1				
Bile Duct Hyperplasia								2			2			2		3				
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct					2															
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular						1						1						2		
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic		2	4	2		1		1				3					2		3	
Focus/Foci, Clear Cell				2						2	1	2	3						3	2
Focus/Foci, Eosinophilic		5	4	5	2	3	3	5	2	4		5	2	4	5	4	5	4	5	3
Focus/Foci, Mixed Cell			4							2						3	3			
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular		3	2	3	1	1	2	4	2	2				2			5	2	2	2
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal															2					
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation								2								5				
Pigment Deposition			2	2		1	2	2	1							3	2			

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	8399	8400	8401	8402	8403	8404	8405	8406	8407	8408	8409	8410	8411	8412	8413	8414	8415	8416	8417	8418
LIVER, CONSENSUS												N								
Hepatocellular Adenoma	P	P		P	P	P	P	P	P	P	P		P	P	P	P	P	P	P	P
Hepatocellular Carcinoma	P								P		P					P				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis				2		2								2						
Bile Duct Hyperplasia			2										2			2				
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic	4								2								3			
Dilated Bile Ducts, Focal		2							1											
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular														1				1		
Fatty Change, Diffuse											2									
Fatty Change, Focal																				
Focus/Foci, Basophilic			2	3		3			4				3	2					2	
Focus/Foci, Clear Cell			2		1		1	3		1				2	2				2	4
Focus/Foci, Eosinophilic		3		5	5	5	1	5	5	4	5		4	3		5	4	5	4	3
Focus/Foci, Mixed Cell			3	2																3
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal															2					
Hepatitis, Suppurative, Multifocal								1												
Hepatocytomegaly, Centrilobular	2	1	2	4		4	2						2	2		2		2	3	1
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal								1			4		2							
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pigment Deposition			1		3		2				2		2			1		1		

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m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	84119	84202	84221	84222	84223	84224	84225	84226	84227	84228	84229	84230	84231	84232	84233	84234	84235	84236	84237	84238
LIVER, CONSENSUS																				
Hepatocellular Adenoma		P			P	P		P	P	P	P		P		P	P	P			P
Hepatocellular Carcinoma						P								P	P		P			
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis						2		2										1		
Bile Duct Hyperplasia						2		2		2	1									
Cellular Atypia, Diffuse																				
Cholangiofibrosis															3					
Cystic Bile Duct								4												
Degeneration, Cystic																				
Dilated Bile Ducts, Focal		2			2					3							1			
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular				2				2	1	2				1		1				
Fatty Change, Diffuse					1															
Fatty Change, Focal						1											2			
Focus/Foci, Basophilic						4		5		3		3	2				2			
Focus/Foci, Clear Cell	1					4		3				3	3			2	2	2		
Focus/Foci, Eosinophilic	3	4	3	4	3	4		5	5	5		4	4	2	5		5	5	3	4
Focus/Foci, Mixed Cell										3		3					3	4	1	
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular	2			3	2	2	1	2	2	3		3	2	2			2	2	2	
Hepatocytomegaly, Diffuse															5					
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal								1							3		3			
Necrosis, Multifocal																				
Oval Cell Proliferation															4					
Pigment Deposition				1		2		2			1			2	3		2			

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	84439	84440	84441	84442	84443	84444	84445	84446	84447	84448	84449	84450	84451	84452	84453	84454	84455	84456	84457	84458
LIVER, CONSENSUS											N		•			X				X
Hepatocellular Adenoma	P	P	P			P	P	P	P	P		P	P	P	P		P	P	P	
Hepatocellular Carcinoma						P														
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis		3					3							2						
Bile Duct Hyperplasia	2											2								
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal		3																		
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular				2											1					
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic				3			4		2					3						
Focus/Foci, Clear Cell					1	2								2				2		
Focus/Foci, Eosinophilic		5	5	3	3	3	5		5	3		5	3	3	5		4	5		
Focus/Foci, Mixed Cell			3				4		3						3			2		
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular				3	2	2			2	1			1	2	3		2			
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal												4			3					
Necrosis, Multifocal																				
Oval Cell Proliferation	2		2				3													
Pigment Deposition	2			2	2				2	1		2		2	2			3		

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	84459	84460	84461	84462	84463	84464	84465	84466	84467	84468	84469	84470	84471	84472	84473	84474	84475	84476	84477	84478
LIVER, CONSENSUS						N														
Hepatocellular Adenoma	P		P	P	P		P	P	P	P	P	P	P	P	P	P	P			P
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia	2																4			
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic										3					3					
Dilated Bile Ducts, Focal																	2			
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular										3				1		1			1	
Fatty Change, Diffuse									1											
Fatty Change, Focal																				
Focus/Foci, Basophilic	2		4								3	3	1		2				2	3
Focus/Foci, Clear Cell	4	2	1						2	3	3	3	1						2	3
Focus/Foci, Eosinophilic	4	4	4	2	2		4	5	2	5	5	5	4	5	4	4		3	4	4
Focus/Foci, Mixed Cell		3									2	4				4				
Hepatitis, Granulomatous, Multifocal								2												
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular			3	1					2	2				2	2	2			1	2
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation	3																			
Pigment Deposition			2	2								2	2					1	1	

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	84479	84480	84481	84482	84483	84484	84485	84486	84487	84488	84489	84490	84491	84492	84493	84494	84495	84496	84497	84498
LIVER, CONSENSUS									N											
Hepatocellular Adenoma		P	P	P	P	P	P	P		P		P	P	P	P	P	P	P	P	P
Hepatocellular Carcinoma		P					P								P					
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis				3						2								2		
Bile Duct Hyperplasia								1		2	1									
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular							2							1						
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Focus/Foci, Basophilic		3		2		1				4								4		2
Focus/Foci, Clear Cell				3			2				1	4		2	3	1		3		2
Focus/Foci, Eosinophilic	2	4	3	5	4	5	4	5		5	2	5	4	3	4	3	5	5	2	5
Focus/Foci, Mixed Cell		3					3								3					1
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular	2	3	3	2		3	3						2	1						
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal										3					2					
Necrosis, Multifocal																				
Oval Cell Proliferation										3										
Pigment Deposition	1	2				1				3									1	

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260 Sacrifice
Female Rat

GROUP A2

ANIMAL	84909	85001	85002	85003	85004	85005	85006	85007	85008	85009	85010	85011	85012	85013	85014	85015	85016	85017	85018
LIVER, CONSENSUS				X								N							
Hepatocellular Adenoma	P	P	P	P		P	P	P	P	P	P			P		P		P	P	
Hepatocellular Carcinoma						P			P											P
Leukemia, Granulocytic																				
Leukemia, Lymphocytic																				
Lymphoma, Malignant																				
Angiectasis						2						1								
Bile Duct Hyperplasia			1												2					
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal			2	2																
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular										1				2			2			
Fatty Change, Diffuse														1				2		
Fatty Change, Focal																				
Focus/Foci, Basophilic		3		2		3					2	3				2		4		
Focus/Foci, Clear Cell		2	3	1																
Focus/Foci, Eosinophilic	4	5	5	5		5	4	4	4	4	4	3		4	3	5	1	5	3	5
Focus/Foci, Mixed Cell	3																		2	
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocytomegaly, Centrilobular		2	2	5			3		2	1		1				4	1	3	1	3
Hepatocytomegaly, Diffuse																				
Hepatocytomegaly, Periportal																				
Leukocytosis																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation			1												2					
Pigment Deposition				2		1		2							1		2			

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

APPENDIX B

Aroclor 1260 Reproduction Study in
Male and Female Sherman Rats
(Linder, et al., 1974)

Males

Group A1- Control
Group A3 - 100 ppm Aroclor 1260

Females

Group A2 - Control
Group A4 - 100 ppm Aroclor 1260

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

SUMMARY INCIDENCE TABLES

SUMMARY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260(7-70) Sacrifice
Male Rat

	GROUP A1	GROUP A3				
LIVER, CONSENSUS (NO. EXAMINED)	(10)	(10)				
Cytoplasmic Inclusions		2				
Fatty Change, Centrilobular						
Fatty Change, Focal						
Focus/Foci, Clear Cell		1				
Focus/Foci, Eosinophilic						
Hepatocytomegaly, Centrilobular		10				
Microgranuloma(s)	3	2				
Pigment, Deposition						

SUMMARY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260(7-70) Sacrifice
Female Rat

	GROUP A2	GROUP A4				
LIVER, CONSENSUS (NO. EXAMINED)	(10)	(10)				
Cytoplasmic Inclusions						
Fatty Change, Centrilobular		2				
Fatty Change, Focal		1				
Focus/Foci, Clear Cell						
Focus/Foci, Eosinophilic		2				
Hepatocytomegaly, Centrilobular		7				
Microgranuloma(s)	5	3				
Pigment, Deposition		4				

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

HISTOPATHOLOGY INCIDENCE TABLES

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260(7-70) Sacrifice
Male Rat

GROUP
A1

ANIMAL	89001	89002	89003	89004	89005	89006	89007	89008	89009	89010
LIVER, CONSENSUS	X	X	X	X			X		X	X
Cytoplasmic Inclusions										
Fatty Change, Centrilobular										
Fatty Change, Focal										
Focus/Foci, Clear Cell										
Focus/Foci, Eosinophilic										
Hepatocytomegaly, Centrilobular										
Microgranuloma(s)					1	1		1		
Pigment, Deposition										

EPL Experimental Pathology Laboratories, Inc.

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P=Present B=Benign M=Malignant
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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260(7-70) Sacrifice
Male Rat

GROUP
A3

ANIMAL	89201	89202	89203	89204	89205	89206	89207	89208	89209	89301
LIVER, CONSENSUS										
Cytoplasmic Inclusions						4		3		
Fatty Change, Centrilobular										
Fatty Change, Focal										
Focus/Foci, Clear Cell					1					
Focus/Foci, Eosinophilic										
Hepatocytomegaly, Centrilobular	2	2	3	4	4	5	3	5	4	3
Microgranuloma(s)		1					1			
Pigment, Deposition										

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260(7-70) Sacrifice
Female Rat

GROUP
A2

ANIMAL	89111	89112	89113	89114	89115	89116	89117	89118	89119	89120
LIVER, CONSENSUS		X		X				X	X	X
Cytoplasmic Inclusions										
Fatty Change, Centrilobular										
Fatty Change, Focal										
Focus/Foci, Clear Cell										
Focus/Foci, Eosinophilic										
Hepatocytomegaly, Centrilobular										
Microgranuloma(s)	1		1		2	1	1			
Pigment, Deposition										

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
CDC/1260(7-70) Sacrifice
Female Rat

GROUP A4

ANIMAL	8931	8932	8933	8934	8935	8936	8937	8938	8939	8940
LIVER, CONSENSUS				X						
Cytoplasmic Inclusions										
Fatty Change, Centrilobular			2				1			
Fatty Change, Focal										1
Focus/Foci, Clear Cell										
Focus/Foci, Eosinophilic		1								2
Hepatocytomegaly, Centrilobular	1	1	4		1	2		1		2
Microgranuloma(s)		1			1				1	
Pigment, Deposition		1				1	1		1	

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Experimental Pathology Laboratories, Inc.

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

APPENDIX C

Aroclor 1260 in Male and Female Spraque-Dawley Rats
(Norback and Weltman, 1985)

Group Control - Control
Group 1260 - 100 ppm for 16 Months Followed by 50 ppm for 8 Months Then Control Diet for 5 Months

SUMMARY INCIDENCE TABLES

SUMMARY INCIDENCE TABLE

PCB Liver Reassessment
UNofWIS/1260 Sacrifice
Male Rat

	GROUP CONTROL	GROUP 1260				
LIVER, CONSENSUS (NO. EXAMINED)	(31)	(42)				
Cholangiocarcinoma						
Hepatocellular Adenoma		4				
Hepatocellular Carcinoma		1				
Leukemia, Granulocytic						
Lymphoma, Malignant						
Abscess						
Adhesions						
Angiectasis		1				
Bile Duct Hyperplasia	6	8				
Cellular Atypia, Diffuse						
Cholangiofibrosis						
Cystic Bile Ducts		1				
Degeneration, Cystic	1	2				
Dilated Bile Ducts, Focal		1				
Fatty Change, Centrilobular		17				
Fatty Change, Diffuse		1				
Fatty Change, Focal	1	1				
Fibrosis, Periportal	2	3				
Focus/Foci, Basophilic	1					
cus/Foci, Clear Cell	4					
ocus/Foci, Eosinophilic	1	16				
Focus/Foci, Mixed Cell		2				
Hepatitis, Granulomatous, Multifocal	1					
Hepatitis, Necrotizing, Multifocal	2	1				
Hepatocellular Hyperplasia						
Hepatocytomegaly, Centrilobular		15				
Necrosis, Centrilobular		7				
Necrosis, Focal	1	1				
Pigment Deposition	1	2				

SUMMARY INCIDENCE TABLE

PCB Liver Reassessment
UNofWIS/1260 Sacrifice
Female Rat

	GROUP CONTROL	GROUP 1260				
LIVER, CONSENSUS (NO. EXAMINED)	(47)	(46)				
Cholangiocarcinoma		3				
Hepatocellular Adenoma	1	29				
Hepatocellular Carcinoma		19				
Leukemia, Granulocytic	1					
Lymphoma, Malignant	1					
Abscess	1					
Adhesions	1					
Angiectasis	1	1				
Bile Duct Hyperplasia	2	19				
Cellular Atypia, Diffuse		1				
Cholangiofibrosis		4				
Cystic Bile Ducts	1	5				
Degeneration, Cystic		3				
Dilated Bile Ducts, Focal	1	2				
Fatty Change, Centrilobular		2				
Fatty Change, Diffuse						
Fatty Change, Focal						
Fibrosis, Periportal		12				
Focus/Foci, Basophilic	2	1				
ocus/Foci, Clear Cell	1					
ocus/Foci, Eosinophilic	5	36				
Focus/Foci, Mixed Cell						
Hepatitis, Granulomatous, Multifocal						
Hepatitis, Necrotizing, Multifocal						
Hepatocellular Hyperplasia		2				
Hepatocytomegaly, Centrilobular		5				
Necrosis, Centrilobular	2	4				
Necrosis, Focal	1	5				
Pigment Deposition	2	13				

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

HISTOPATHOLOGY INCIDENCE TABLES

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HISTOPATHOLOGY INCIDENCE TABLE

GROUP
CONTROL

Liver Reassessment
UNofWIS/1260 Sacrifice
Male Rat

ANIMAL	20	23	24	25	26	28	29	30	33	34	35	37	38	40	41	42	43	46	51	52
LIVER, CONSENSUS	X	X		X	X	X	X	A	X						X	X		X	X	
Cholangiocarcinoma																				
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Lymphoma, Malignant																				
Abscess																				
Adhesions																				
Angiectasis																				
Bile Duct Hyperplasia			1							2	1		1							
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Ducts																				
Degeneration, Cystic											1									
Dilated Bile Ducts, Focal																				
tty Change, Centrilobular																				
atty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal			1							1										
Focus/Foci, Basophilic											1									
Focus/Foci, Clear Cell											1		1	1						1
Focus/Foci, Eosinophilic										1										
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																	2			
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Necrosis, Centrilobular																				
Necrosis, Focal												3								
Pigment Deposition																				

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

) Liver Reassessment
UNofWIS/1260 Sacrifice
Male Rat

GROUP
CONTROL

ANIMAL	56	60	61	66	69	71	72	74	76	78	79									
LIVER, CONSENSUS	A		X		X		X	X		X	X									
Cholangiocarcinoma																				
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Lymphoma, Malignant																				
Abscess																				
Adhesions																				
Angiectasis																				
Bile Duct Hyperplasia						2			1											
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Ducts																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
atty Change, Centrilobular																				
atty Change, Diffuse																				
Fatty Change, Focal		1																		
Fibrosis, Periportal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic																				
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal						2														
Hepatitis, Necrotizing, Multifocal				3																
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Pigment Deposition						3														

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
UNofWIS/1260 Sacrifice
Male Rat

GROUP 1260

ANIMAL	0	166	169	173	176	178	182	185	187	190	191	193	194	195	196	197	199	201	202	204
LIVER, CONSENSUS					X	A							A	X						
Cholangiocarcinoma																				
Hepatocellular Adenoma																			P	
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Lymphoma, Malignant																				
Abscess																				
Adhesions																				
Angiectasis																				
Bile Duct Hyperplasia			2												1				2	
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Ducts																				
Degeneration, Cystic				1																
Dilated Bile Ducts, Focal															1					
Fatty Change, Centrilobular	2						2			1	1	1			1			1		
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal		3	2																	
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic			2	4					1	1		2			2				4	2
Focus/Foci, Mixed Cell																			2	
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal		3																		
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular	3							1	2		3	2			2	2	2	2	2	
Necrosis, Centrilobular							4			2										
Necrosis, Focal																				
Pigment Deposition			2								2									

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

...3 Liver Reassessment
UNofWIS/1260 Sacrifice
Male Rat

GROUP 1260

ANIMAL	206	207	210	211	214	216	217	220	221	222	223	225	226	230	231	232	233	234	235	236
LIVER, CONSENSUS	X				N	X		A											N	A
Cholangiocarcinoma																				
Hepatocellular Adenoma												P	P							
Hepatocellular Carcinoma																	P			
Leukemia, Granulocytic																				
Lymphoma, Malignant																				
Abscess																				
Adhesions																				
Angiectasis																				3
Bile Duct Hyperplasia							2				2			2						
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Ducts													4							
Degeneration, Cystic				1				-												
Dilated Bile Ducts, Focal																				
atty Change, Centrilobular		2	2	2					2		2			2	1	2		1		2
fatty Change, Diffuse																				
Fatty Change, Focal													1							
Fibrosis, Periportal											2									
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic									1	1	3	3	1	1	2			1		
Focus/Foci, Mixed Cell											2									
Hepatitis, Granulomatous,														.						
Multifocal																				
Hepatitis, Necrotizing,																				
Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly,																				
Centrilobular			3								2				4					
Necrosis, Centrilobular							1		1				3	2						2
Necrosis, Focal														2						
Pigment Deposition																				

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Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
UNofWIS/1260 Sacrifice
Male Rat

	GROUP 1260																					
ANIMAL	185B	189A	189B	212A	212B																	
LIVER, CONSENSUS	N																					
Cholangiocarcinoma																						
Hepatocellular Adenoma			P																			
Hepatocellular Carcinoma																						
Leukemia, Granulocytic																						
Lymphoma, Malignant																						
Abscess																						
Adhesions																						
Angiectasis																						
Bile Duct Hyperplasia		1	2																			
Cellular Atypia, Diffuse																						
Cholangiofibrosis																						
Cystic Bile Ducts																						
Degeneration, Cystic																						
Dilated Bile Ducts, Focal																						
Fatty Change, Centrilobular																						
Fatty Change, Diffuse					1																	
Fatty Change, Focal																						
Fibrosis, Periportal																						
Focus/Foci, Basophilic																						
Focus/Foci, Clear Cell																						
Focus/Foci, Eosinophilic																						
Focus/Foci, Mixed Cell																						
Hepatitis, Granulomatous, Multifocal																						
Hepatitis, Necrotizing, Multifocal																						
Hepatocellular Hyperplasia																						
Hepatocytomegaly, Centrilobular		2		1																		
Necrosis, Centrilobular																						
Necrosis, Focal																						
Pigment Deposition																						

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

Liver Reassessment
UNofWIS/1260 Sacrifice
Female Rat

GROUP
CONTROL

ANIMAL	16	23	24	25	26	27	28	29	32	34	35	36	37	38	40	42	43	44	45	46
LIVER, CONSENSUS	X	X	X	X	X		X			X	X	X		X		X	X	X		
Cholangiocarcinoma																				
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Lymphoma, Malignant																				
Abscess								P												
Adhesions								P												
Angiectasis																				
Bile Duct Hyperplasia						2														
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Ducts																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
tty Change, Centrilobular																				
atty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic																			2	3
Focus/Foci, Clear Cell									1											
Focus/Foci, Eosinophilic						2							2						1	
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Necrosis, Centrilobular																				
Necrosis, Focal															2					
Pigment Deposition								1												

EPL | Experimental Pathology Laboratories, Inc.

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
UNofWIS/1260 Sacrifice
Female Rat

GROUP
CONTROL

ANIMAL	48	50	51	52	53	55	56	58	59	60	62	65	67	68	70	71	72	74	75	76
LIVER, CONSENSUS		X	X		X			X	X		X	X	X	X		X	X	A	X	X
Cholangiocarcinoma																				
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Leukemia, Granulocytic															P					
Lymphoma, Malignant							P													
Abscess																				
Adhesions																				
Angiectasis						2														
Bile Duct Hyperplasia																				
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Ducts																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal	1																			
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic						3				2										
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Necrosis, Centrilobular				2		3														
Necrosis, Focal																				
Pigment Deposition							2													

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

Liver Reassessment
UNofWIS/1260 Sacrifice
Female Rat

GROUP
CONTROL

ANIMAL	77	78	80	30A	30B	31A	31B													
LIVER, CONSENSUS			X	X		X	X													
Cholangiocarcinoma																				
Hepatocellular Adenoma		P																		
Hepatocellular Carcinoma																				
Leukemia, Granulocytic																				
Lymphoma, Malignant																				
Abscess																				
Adhesions																				
Angiectasis																				
Bile Duct Hyperplasia					2															
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				
Cystic Bile Ducts	4																			
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
ıtty Change, Centrilobular																				
atty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic																				
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Pigment Deposition																				

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P=Present B=Benign M=Malignant
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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
UNofWIS/1260 Sacrifice
Female Rat

GROUP 1260

ANIMAL	0	166	170	177	178	182	187	188	189	190	191	192	193	195	196	197	198	201	202	203
LIVER, CONSENSUS			N																	
Cholangiocarcinoma						P														
Hepatocellular Adenoma					P	P	P			P	P			P		P	P	P	P	P
Hepatocellular Carcinoma	P	P										P	P				P	P		
Leukemia, Granulocytic																				
Lymphoma, Malignant																				
Abscess																				
Adhesions																				
Angiectasis																				
Bile Duct Hyperplasia	3			2	3	3			3	3	2		2	2		2			1	
Cellular Atypia, Diffuse																				
Cholangiofibrosis																				4
Cystic Bile Ducts											4					4				
Degeneration, Cystic										5						-				
Dilated Bile Ducts, Focal																2				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal	2			2	3					2			2	4		2	3			
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic	2				2	5	3	3	4	5	5	5	4	3	3		3	5	4	3
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia										P				P						
Hepatocytomegaly, Centrilobular									2						2					
Necrosis, Centrilobular							2												2	
Necrosis, Focal				5		3							3						4	
Pigment Deposition					2								1	2	1	2				

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Experimental Pathology Laboratories, Inc.

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HISTOPATHOLOGY INCIDENCE TABLE

Liver Reassessment
UNofWIS/1260 Sacrifice
Female Rat

	GROUP 1260																		
ANIMAL	204	205	206	207	208	209	210	211	212	213	214	216	217	219	220	221	222	224	225	226
LIVER, CONSENSUS																				
Cholangiocarcinoma								P												
Hepatocellular Adenoma	P	P	P	P	P	P	P	P	P	P	P	P		P				P	P	P
Hepatocellular Carcinoma			P							P	P				P	P	P		P	
Leukemia, Granulocytic																				
Lymphoma, Malignant																				
Abscess																				
Adhesions																				
Angiectasis																		3		
Bile Duct Hyperplasia									3				3		4	2		3	2	
Cellular Atypia, Diffuse																				4
Cholangiofibrosis						4			3											
Cystic Bile Ducts			3								3							3		
Degeneration, Cystic														2						2
Dilated Bile Ducts, Focal										3										
Fatty Change, Centrilobular		2												1						
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal															3				2	
Focus/Foci, Basophilic																		3		
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic	5	4	4	3	4	4		3	5		4	3	5	5	2		3	2		2
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular									3					2				3		
Necrosis, Centrilobular								1												4
Necrosis, Focal																				
Pigment Deposition						2		2	3				2		3		2		2	

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Experimental Pathology Laboratories, Inc.

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
UNofWIS/1260 Sacrifice
Female Rat

GROUP 1260

ANIMAL	227	228	229	231	233	235	236
LIVER, CONSENSUS							
Cholangiocarcinoma	P						
Hepatocellular Adenoma				P	P		
Hepatocellular Carcinoma	P	P	P	P		P	P
Leukemia, Granulocytic							
Lymphoma, Malignant							
Abscess							
Adhesions							
Angiectasis							
Bile Duct Hyperplasia			3	3			
Cellular Atypia, Diffuse							
Cholangiofibrosis							4
Cystic Bile Ducts							
Degeneration, Cystic							
Dilated Bile Ducts, Focal							
Fatty Change, Centrilobular							
Fatty Change, Diffuse							
Fatty Change, Focal							
Fibrosis, Periportal	3		3				
Focus/Foci, Basophilic							
Focus/Foci, Clear Cell							
Focus/Foci, Eosinophilic	5			5	4	3	
Focus/Foci, Mixed Cell							
Hepatitis, Granulomatous, Multifocal							
Hepatitis, Necrotizing, Multifocal							
Hepatocellular Hyperplasia							
Hepatocytomegaly, Centrilobular							
Necrosis, Centrilobular							
Necrosis, Focal	2						
Pigment Deposition	3						

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

APPENDIX D

National Cancer Institute (NCI) 1977 Aroclor 1254 Study
in Male and Female Fischer 344 Rats
(NCI, 1977)

Males

Group 01-1645 - Control
Group 01-1639 - 25 ppm Aroclor
Group 01-1641 - 50 ppm Aroclor
Group 01-1643 - 100 ppm Aroclor

Females

Group 01-1646 - Control
Group 01-1640 - 25 ppm Aroclor
Group 01-1642 - 50 ppm Aroclor
Group 01-1644 - 100 ppm Aroclor

SUMMARY INCIDENCE TABLES

SUMMARY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Male Rat

	GROUP 01-1645	GROUP 01-1639	GROUP 01-1641	GROUP 01-1643		
LIVER, CONSENSUS (NO. EXAMINED)	(24)	(24)	(24)	(23)		
Hepatocellular Adenoma		1	1	1		
Hepatocellular Carcinoma				2		
Mononuclear Cell Leukemia	2	3	5	7		
Bile Duct Hyperplasia	2	1	2			
Cystic Bile Duct						
Extramedullary Hematopoiesis						
Fatty Change, Centrilobular		1				
Fatty Change, Diffuse		1				
Fatty Change, Focal				1		
Fibrosis, Periportal						
Focus/Foci, Basophilic		1		7		
Focus/Foci, Clear Cell						
Focus/Foci, Eosinophilic		4	5	4		
Focus/Foci, Mixed Cell		2	4	8		
Hepatitis, Granulomatous,						
Focal				1		
Hepatitis, Granulomatous,						
Multifocal			1	2		
Hepatitis, Necrotizing,						
Multifocal				1		
Hepatocellular Hyperplasia				1		
Hepatocytomegaly,						
Centrilobular		3	3	3		
Hepatocytomegaly, Diffuse				1		
Necrosis, Centrilobular	1	1				
Necrosis, Focal				3		
Oval Cell Proliferation				1		
Pigment Deposition			2	2		

SUMMARY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Female Rat

	GROUP 01-1646	GROUP 01-1640	GROUP 01-1642	GROUP 01-1644		
LIVER, CONSENSUS (NO. EXAMINED)	(23)	(24)	(24)	(24)		
Hepatocellular Adenoma		1	2	1		
Hepatocellular Carcinoma						
Mononuclear Cell Leukemia	3	7	4	5		
Bile Duct Hyperplasia		3	4	1		
Cystic Bile Duct				1		
Extramedullary Hematopoiesis		1				
Fatty Change, Centrilobular				2		
Fatty Change, Diffuse						
Fatty Change, Focal				3		
Fibrosis, Periportal		1				
Focus/Foci, Basophilic	2	2		4		
Focus/Foci, Clear Cell			1	2		
Focus/Foci, Eosinophilic		10	13	6		
Focus/Foci, Mixed Cell		1		5		
Hepatitis, Granulomatous,						
Focal	1	1				
Hepatitis, Granulomatous,						
Multifocal	2	3	11	14		
Hepatitis, Necrotizing,						
Multifocal						
Hepatocellular Hyperplasia		1				
Hepatocytomegaly,						
Centrilobular		3	1	3		
Hepatocytomegaly, Diffuse		1	2			
Necrosis, Centrilobular	2	3	1			
Necrosis, Focal						
Oval Cell Proliferation				2		
Pigment Deposition	3	16	16	18		

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HISTOPATHOLOGY INCIDENCE TABLES

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HISTOPATHOLOGY INCIDENCE TABLE

, Liver Reassessment
NTP/1254 Sacrifice
Male Rat

GROUP
01-1645

ANIMAL	011	012	013	021	022	023	031	032	033	041	042	043	051	052	053	061	062	063	071	072
LIVER, CONSENSUS		X	X	X			X	X	X	X	X	X	X	X	X	X	X		X	X
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Mononuclear Cell Leukemia	P																	P		
Bile Duct Hyperplasia					1	2														
Cystic Bile Duct																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic																				
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Focal																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Necrosis, Centrilobular																		2		
Necrosis, Focal																				
Oval Cell Proliferation																				
Pigment Deposition																				

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Male Rat

	GROUP 01-1645																							
ANIMAL	073	081	082	083																				
LIVER, CONSENSUS	X	X	X	X																				
Hepatocellular Adenoma																								
Hepatocellular Carcinoma																								
Mononuclear Cell Leukemia																								
Bile Duct Hyperplasia																								
Cystic Bile Duct																								
Extramedullary Hematopoiesis																								
Fatty Change, Centrilobular																								
Fatty Change, Diffuse																								
Fatty Change, Focal																								
Fibrosis, Periportal																								
Focus/Foci, Basophilic																								
Focus/Foci, Clear Cell																								
Focus/Foci, Eosinophilic																								
Focus/Foci, Mixed Cell																								
Hepatitis, Granulomatous, Focal																								
Hepatitis, Granulomatous, Multifocal																								
Hepatitis, Necrotizing, Multifocal																								
Hepatocellular Hyperplasia																								
Hepatocytomegaly, Centrilobular																								
Hepatocytomegaly, Diffuse																								
Necrosis, Centrilobular																								
Necrosis, Focal																								
Oval Cell Proliferation																								
Pigment Deposition																								

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Male Rat

GROUP
01-1639

ANIMAL	011	012	013	021	022	023	031	032	033	041	042	043	051	052	053	061	062	063	071	072
LIVER, CONSENSUS					X	X			X	X	X	X	X	X	X	X				
Hepatocellular Adenoma		P																		
Hepatocellular Carcinoma																				
Mononuclear Cell Leukemia		P		P													P			
Bile Duct Hyperplasia																				1
Cystic Bile Duct																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular								1												
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic		3					2											2	2	
Focus/Foci, Mixed Cell	2							2												
Hepatitis, Granulomatous, Focal																				
Hepatitis, Granulomatous, Multifocal																				
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular			1				1													
Hepatocytomegaly, Diffuse																				
Necrosis, Centrilobular				3																
Necrosis, Focal																				
Oval Cell Proliferation																				
Pigment Deposition																				

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Male Rat

GROUP
01-1639

ANIMAL	073	081	082	083
LIVER, CONSENSUS		X		X
Hepatocellular Adenoma				
Hepatocellular Carcinoma				
Mononuclear Cell Leukemia				
Bile Duct Hyperplasia				
Cystic Bile Duct				
Extramedullary Hematopoiesis				
Fatty Change, Centrilobular				
Fatty Change, Diffuse	1			
Fatty Change, Focal				
Fibrosis, Periportal				
Focus/Foci, Basophilic			1	
Focus/Foci, Clear Cell				
Focus/Foci, Eosinophilic				
Focus/Foci, Mixed Cell				
Hepatitis, Granulomatous, Focal				
Hepatitis, Granulomatous, Multifocal				
Hepatitis, Necrotizing, Multifocal				
Hepatocellular Hyperplasia				
Hepatocytomegaly, Centrilobular			1	
Hepatocytomegaly, Diffuse				
Necrosis, Centrilobular				
Necrosis, Focal				
Oval Cell Proliferation				
Pigment Deposition				

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Male Rat

GROUP
01-1641

ANIMAL	171	172	173	181	182	183	191	192	193	201	202	203	211	212	213	221	222	223	231	232
LIVER, CONSENSUS		X					X	X				X	X				X		X	
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Mononuclear Cell Leukemia			P			P				P	P									
Bile Duct Hyperplasia											2									
Cystic Bile Duct																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic				2					1	2					2			2		
Focus/Foci, Mixed Cell			3		3											2				
Hepatitis, Granulomatous, Focal																				
Hepatitis, Granulomatous, Multifocal																				1
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular					1									1				2		
Hepatocytomegaly, Diffuse																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Oval Cell Proliferation																				
Pigment Deposition	1			1																

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Male Rat

	GROUP 01-1641			
ANIMAL	233	241	242	243
LIVER, CONSENSUS			X	
Hepatocellular Adenoma				P
Hepatocellular Carcinoma				
Mononuclear Cell Leukemia	P			
Bile Duct Hyperplasia				
Cystic Bile Duct				
Extramedullary Hematopoiesis				
Fatty Change, Centrilobular				
Fatty Change, Diffuse				
Fatty Change, Focal				
Fibrosis, Periportal				
Focus/Foci, Basophilic				
Focus/Foci, Clear Cell				
Focus/Foci, Eosinophilic				
Focus/Foci, Mixed Cell		2		
Hepatitis, Granulomatous, Focal				
Hepatitis, Granulomatous, Multifocal				
Hepatitis, Necrotizing, Multifocal				
Hepatocellular Hyperplasia				
Hepatocytomegaly, Centrilobular				
Hepatocytomegaly, Diffuse				
Necrosis, Centrilobular				
Necrosis, Focal				
Oval Cell Proliferation				
Pigment Deposition				

EPL
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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Male Rat

GROUP
01-1643

ANIMAL	331	332	333	341	342	343	351	352	353	361	362	363	371	372	373	381	382	383	391	392
LIVER, CONSENSUS									X		X									
Hepatocellular Adenoma							P													
Hepatocellular Carcinoma															P	P				
Mononuclear Cell Leukemia					P	P	P	P				P					P			P
Bile Duct Hyperplasia																				
Cystic Bile Duct																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic	1			1											1		3		2	2
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic					3	3								4						3
Focus/Foci, Mixed Cell		3					4			3		3	2	2				2		
Hepatitis, Granulomatous, Focal																				2
Hepatitis, Granulomatous, Multifocal			3	1																
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia					P															
Hepatocytomegaly, Centrilobular				1									2					2		
Hepatocytomegaly, Diffuse																				2
Necrosis, Centrilobular																				
Necrosis, Focal					3		1									3				
Oval Cell Proliferation																			2	
Pigment Deposition			3																1	

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Male Rat

	GROUP 01-1643			
ANIMAL	3 9 3	4 0 1	4 0 2	4 0 3
LIVER, CONSENSUS	N		X	
Hepatocellular Adenoma				
Hepatocellular Carcinoma				
Mononuclear Cell Leukemia				
Bile Duct Hyperplasia				
Cystic Bile Duct				
Extramedullary Hematopoiesis				
Fatty Change, Centrilobular				
Fatty Change, Diffuse				
Fatty Change, Focal				1
Fibrosis, Periportal				
Focus/Foci, Basophilic				1
Focus/Foci, Clear Cell				
Focus/Foci, Eosinophilic				
Focus/Foci, Mixed Cell				2
Hepatitis, Granulomatous, Focal				
Hepatitis, Granulomatous, Multifocal				
Hepatitis, Necrotizing, Multifocal		2		
Hepatocellular Hyperplasia				
Hepatocytomegaly, Centrilobular				
Hepatocytomegaly, Diffuse				
Necrosis, Centrilobular				
Necrosis, Focal				
Oval Cell Proliferation				
Pigment Deposition				

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

P08 Liver Reassessment
NTP/1254 Sacrifice
Female Rat

GROUP
01-1646

ANIMAL	091	092	093	101	102	103	111	112	113	121	122	123	131	132	133	141	142	143	151	152
LIVER, CONSENSUS	X	X	X	X	X			X	X		X	X	N		X	X	X	X	X	
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Mononuclear Cell Leukemia						P	P													
Bile Duct Hyperplasia																				
Cystic Bile Duct																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic										1										1
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic																				
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Focal																				
Hepatitis, Granulomatous, Multifocal														2						1
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hepatocytomegaly, Diffuse																				
Necrosis, Centrilobular							2													
Necrosis, Focal																				
Oval Cell Proliferation																				
Pigment Deposition							3							1						1

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Female Rat

	GROUP 01-1646																							
ANIMAL	153	161	162	163																				
LIVER, CONSENSUS	X	X																						
Hepatocellular Adenoma																								
Hepatocellular Carcinoma																								
Mononuclear Cell Leukemia				P																				
Bile Duct Hyperplasia																								
Cystic Bile Duct																								
Extramedullary Hematopoiesis																								
Fatty Change, Centrilobular																								
Fatty Change, Diffuse																								
Fatty Change, Focal																								
Fibrosis, Periportal																								
Focus/Foci, Basophilic																								
Focus/Foci, Clear Cell																								
Focus/Foci, Eosinophilic																								
Focus/Foci, Mixed Cell																								
Hepatitis, Granulomatous, Focal			1																					
Hepatitis, Granulomatous, Multifocal																								
Hepatitis, Necrotizing, Multifocal																								
Hepatocellular Hyperplasia																								
Hepatocytomegaly, Centrilobular																								
Hepatocytomegaly, Diffuse																								
Necrosis, Centrilobular				2																				
Necrosis, Focal																								
Oval Cell Proliferation																								
Pigment Deposition																								

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant .
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Female Rat

GROUP
01-1640

ANIMAL	091	092	093	101	102	103	111	112	113	121	122	123	131	132	133	141	142	143	151	152
LIVER, CONSENSUS																				
Hepatocellular Adenoma		P																		
Hepatocellular Carcinoma																				
Mononuclear Cell Leukemia						P		P			P		P			P	P			
Bile Duct Hyperplasia											1	1	2							
Cystic Bile Duct																				
Extramedullary Hematopoiesis																		2		
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal											1									
Focus/Foci, Basophilic																		1	4	
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic		2			2	2	3	3			3			2					2	2
Focus/Foci, Mixed Cell			2																	
Hepatitis, Granulomatous, Focal									2											
Hepatitis, Granulomatous, Multifocal															2		2			
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia								P												
Hepatocytomegaly, Centrilobular														1		3			1	
Hepatocytomegaly, Diffuse	1																			
Necrosis, Centrilobular								2					2					1		
Necrosis, Focal																				
Oval Cell Proliferation																				
Pigment Deposition		1	1	1	2				2	1	2	1	2				1	1	2	

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Female Rat

	GROUP 01-1640																							
ANIMAL	153	161	162	163																				
LIVER, CONSENSUS																								
Hepatocellular Adenoma																								
Hepatocellular Carcinoma																								
Mononuclear Cell Leukemia		P																						
Bile Duct Hyperplasia																								
Cystic Bile Duct																								
Extramedullary Hematopoiesis																								
Fatty Change, Centrilobular																								
Fatty Change, Diffuse																								
Fatty Change, Focal																								
Fibrosis, Periportal																								
Focus/Foci, Basophilic																								
Focus/Foci, Clear Cell																								
Focus/Foci, Eosinophilic				2																				
Focus/Foci, Mixed Cell																								
Hepatitis, Granulomatous, Focal																								
Hepatitis, Granulomatous, Multifocal	2																							
Hepatitis, Necrotizing, Multifocal																								
Hepatocellular Hyperplasia																								
Hepatocytomegaly, Centrilobular																								
Hepatocytomegaly, Diffuse																								
Necrosis, Centrilobular																								
Necrosis, Focal																								
Oval Cell Proliferation																								
Pigment Deposition	2	2	2	1																				

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m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Female Rat

GROUP
01-1642

ANIMAL	251	252	253	261	262	263	271	272	273	281	282	283	291	292	293	301	302	303	311	312
LIVER, CONSENSUS											X									
Hepatocellular Adenoma														P						
Hepatocellular Carcinoma																				
Mononuclear Cell Leukemia	P												P	P		P				
Bile Duct Hyperplasia						1								3						
Cystic Bile Duct																				
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																	1			
Focus/Foci, Eosinophilic	1			2	2	2	2	1						3	2	2		4		
Focus/Foci, Mixed Cell																				
Hepatitis, Granulomatous, Focal																				
Hepatitis, Granulomatous, Multifocal		2						1	2	1		2						3	2	3
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																3				
Hepatocytomegaly, Diffuse									2											
Necrosis, Centrilobular			2																	
Necrosis, Focal																				
Oval Cell Proliferation																				
Pigment Deposition		1		1				2	2	1		2	2	2	1	2	2	3	3	1

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Female Rat

	GROUP 01-1642																						
ANIMAL	3 1 3	3 2 1	3 2 2	3 2 3																			
LIVER, CONSENSUS																							
Hepatocellular Adenoma	P																						
Hepatocellular Carcinoma																							
Mononuclear Cell Leukemia																							
Bile Duct Hyperplasia	2	2																					
Cystic Bile Duct																							
Extramedullary Hematopoiesis																							
Fatty Change, Centrilobular																							
Fatty Change, Diffuse																							
Fatty Change, Focal																							
Fibrosis, Periportal																							
Focus/Foci, Basophilic																							
Focus/Foci, Clear Cell																							
Focus/Foci, Eosinophilic	5	2	3																				
Focus/Foci, Mixed Cell																							
Hepatitis, Granulomatous, Focal																							
Hepatitis, Granulomatous, Multifocal		3	1	4																			
Hepatitis, Necrotizing, Multifocal																							
Hepatocellular Hyperplasia																							
Hepatocytomegaly, Centrilobular																							
Hepatocytomegaly, Diffuse		3																					
Necrosis, Centrilobular																							
Necrosis, Focal																							
Oval Cell Proliferation																							
Pigment Deposition	1		2																				

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Female Rat

GROUP
01-1644

ANIMAL	411	412	413	421	422	423	431	432	433	441	442	443	451	452	453	461	462	463	471	472
LIVER, CONSENSUS																				
Hepatocellular Adenoma																			P	
Hepatocellular Carcinoma																				
Mononuclear Cell Leukemia					P		P	P									P			
Bile Duct Hyperplasia ~														2						
Cystic Bile Duct																		1		
Extramedullary Hematopoiesis																				
Fatty Change, Centrilobular				1												2				
Fatty Change, Diffuse																				
Fatty Change, Focal										1			1					2		
Fibrosis, Periportal																				
Focus/Foci, Basophilic						2												1		
Focus/Foci, Clear Cell									3					1						
Focus/Foci, Eosinophilic			3		1	.	2		1	3					1					
Focus/Foci, Mixed Cell				4								2						2		3
Hepatitis, Granulomatous, Focal																				
Hepatitis, Granulomatous, Multifocal			1	1		1			2	1		1		2	1	2		3		1
Hepatitis, Necrotizing, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular							2					.	1							
Hepatocytomegaly, Diffuse																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Oval Cell Proliferation																				
Pigment Deposition	2	2	2	3			2		2	2	1	2	2	2	2	2	1	2		2

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=[illegible]

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
NTP/1254 Sacrifice
Female Rat

	GROUP 01-1644			
ANIMAL	473	481	482	483
LIVER, CONSENSUS				
Hepatocellular Adenoma				
Hepatocellular Carcinoma				
Mononuclear Cell Leukemia	P			
Bile Duct Hyperplasia				
Cystic Bile Duct				
Extramedullary Hematopoiesis				
Fatty Change, Centrilobular				
Fatty Change, Diffuse				
Fatty Change, Focal				
Fibrosis, Periportal				
Focus/Foci, Basophilic		1		1
Focus/Foci, Clear Cell				
Focus/Foci, Eosinophilic				
Focus/Foci, Mixed Cell			3	
Hepatitis, Granulomatous, Focal				
Hepatitis, Granulomatous, Multifocal		2	1	2
Hepatitis, Necrotizing, Multifocal				
Hepatocellular Hyperplasia				
Hepatocytomegaly, Centrilobular				1
Hepatocytomegaly, Diffuse				
Necrosis, Centrilobular				
Necrosis, Focal				
Oval Cell Proliferation		1	2	
Pigment Deposition		3	2	

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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

APPENDIX E

Clophen A 60 in Male Wistar Rats
(Schaeffer, et al., 1984)
and Clophen A 30 in Male Wistar Rats
(Schaeffer, et al., 1984)

Group CO - Control
Group 30 - 100 ppm Clophen A 30
Group 60 - 100 ppm Clophen A 60

SUMMARY INCIDENCE TABLES

SUMMARY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

	GROUP CO	GROUP 30	GROUP 60			
LIVER, CONSENSUS (NO. EXAMINED)	(120)	(128)	(125)			
Cholangiocarcinoma	1					
Hepatocellular Adenoma	6	14	85			
Hepatocellular Carcinoma	2	2	67			
Histiocytic Sarcoma			2			
Lymphoma, Malignant	3	1	1			
Angiectasis	2	6	7			
Bile Duct Hyperplasia	112	110	103			
Cholangiofibrosis			1			
Cystic Bile Duct		2	2			
Degeneration, Cystic	9	5	6			
Dilated Bile Ducts, Focal	9	7	7			
Fatty Change, Centrilobular	5	2	1			
Fatty Change, Diffuse	4		1			
Fatty Change, Focal	4	1	2			
Fatty Change, Periportal	1	1	2			
Fibrosis, Centrilobular	1					
Fibrosis, Periportal	51	19	15			
Focus/Foci, Basophilic	2	15	4			
Focus/Foci, Clear Cell	7	39	28			
cus/Foci, Eosinophilic	51	98	101			
ocus/Foci, Mixed Cell	7	49	28			
Hepatitis, Necrotizing,						
Multifocal		1				
Hepatitis, Suppurative,						
Multifocal	1		1			
Hepatocellular Hyperplasia		1	1			
Hepatocytomegaly,						
Centrilobular	1	2	2			
Hyperplasia, Focal			1			
Necrosis, Centrilobular	3	1	2			
Necrosis, Focal	3	4	21			
Necrosis, Multifocal	1					
Oval Cell Proliferation			1			
Pericholangitis, Granulomatous	1					
Pigment Deposition	1		4			

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

HISTOPATHOLOGY INCIDENCE TABLES

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HISTOPATHOLOGY INCIDENCE TABLE

Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP CO

ANIMAL	78/27	78/33	77/68/4	77/70/0	77/74/6	77/79/6	77/82/1	77/96/8	78/10/4	78/11/6	78/23/5	78/24/0	78/27/5	78/28/8	78/30/5	78/30/6	78/33/2	78/56/6	78/59/9	78/67/2
LIVER, CONSENSUS		X				X	N											N		
Cholangiocarcinoma																				P
Hepatocellular Adenoma																				
Hepatocellular Carcinoma											P									P
Histiocytic Sarcoma																				
Lymphoma, Malignant	P																			
Angiectasis																				
Bile Duct Hyperplasia	3		2	2	3			2	1	2	1	3	2	2	2	2	1		5	
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal	1			1											1					
Fatty Change, Centrilobular													3							
Fatty Change, Diffuse																				
Fatty Change, Focal																	1			
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal	2								1	1		1		2						
Focus/Foci, Basophilic																				4
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic	1								2			2	2		3	1	1			
Focus/Foci, Mixed Cell													4							
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				3
Hyperplasia, Focal																				
Necrosis, Centrilobular											2									
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition												2								

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m=missing one paired organ u=moribund sac./death

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

	GROUP CO																				
ANIMAL	78/69 1	78/81 1	78/82 2	78/82 3	78/90 6	78/91 8	78/92 0	78/98 7	78/99 1	77/00 3	77/00 9	77/01 6	77/02 2	77/03 4	77/03 7	77/06 5	77/13 7	77/17 8	77/17 7	77/18 1	77/19 2
LIVER, CONSENSUS			N	N						N	N						N	N	N		
Cholangiocarcinoma																					
Hepatocellular Adenoma															P						
Hepatocellular Carcinoma																					
Histiocytic Sarcoma																					
Lymphoma, Malignant																					
Angiectasis																					
Bile Duct Hyperplasia	3	3			4	4	3	4	2				2	2	1					2	5
Cholangiofibrosis																					
Cystic Bile Duct																					
Degeneration, Cystic																					
Dilated Bile Ducts, Focal																					
Fatty Change, Centrilobular																					
Fatty Change, Diffuse																					3
Fatty Change, Focal																					
Fatty Change, Periportal																					
Fibrosis, Centrilobular																					5
Fibrosis, Periportal	2				3	2		3	1											2	
Focus/Foci, Basophilic																					
Focus/Foci, Clear Cell																					
Focus/Foci, Eosinophilic							2		2			2		2							
Focus/Foci, Mixed Cell																					
Hepatitis, Necrotizing, Multifocal																					
Hepatitis, Suppurative, Multifocal																					
Hepatocellular Hyperplasia																					
Hepatocytomegaly, Centrilobular																					
Hyperplasia, Focal																					
Necrosis, Centrilobular																					5
Necrosis, Focal	1																				
Necrosis, Multifocal																					4
Oval Cell Proliferation																					
Pericholangitis, Granulomatous																					
Pigment Deposition																					

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP CO

ANIMAL	77/19 23	78/10 01	78/10 03	78/10 25	78/10 74	78/10 98	78/10 99	78/11 32	78/11 63	78/11 65	78/11 93	78/12 19	78/12 27	78/12 52	78/12 64	78/12 65	78/12 71	78/13 22	78/13 24	78/13 37
LIVER, CONSENSUS																		X		
Cholangiocarcinoma																				
Hepatocellular Adenoma																				
Hepatocellular Carcinoma																				
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia	2	2	3	2	2	3	2	3	2		3	2	2	2	3	4	3		3	4
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic				1						2										
Dilated Bile Ducts, Focal		2				2														
Fatty Change, Centrilobular												3			2					
Fatty Change, Diffuse																				
Fatty Change, Focal										2										
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal	2						1		2			1	2		2		2			2
Focus/Foci, Basophilic						1														
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic		3		3	2	2				3					3					
Focus/Foci, Mixed Cell										2										
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular									2											
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

EPL | Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP CO

ANIMAL	78/13/38	78/13/53	78/13/77	78/13/81	78/14/39	78/14/07	78/14/53	78/14/72	78/14/82	78/14/94	78/14/95	78/15/02	78/15/19	78/15/22	78/15/29	78/15/31	78/15/36	78/15/37	78/15/79	78/15/55
LIVER, CONSENSUS					A															
Cholangiocarcinoma																				
Hepatocellular Adenoma																P				
Hepatocellular Carcinoma																				
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia	4	2	3	3	4	2	1	2	3	3	1	3	1	4	3	3	2	3	1	2
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																3				
Fatty Change, Centrilobular													2				3			
Fatty Change, Diffuse			2						1											
Fatty Change, Focal																5				
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal	2		2	2	2	2		1	1	2	2			2		1	1	2		2
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic		3													2				2	2
Focus/Foci, Mixed Cell																				
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

EPL | Experimental Pathology Laboratories, Inc.

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP CO

ANIMAL	78/1155 58	78/1155 59	78/1157 59	78/1158 82	78/1158 83	78/1158 85	78/1158 90	78/1159 91	78/1159 92	78/1160 63	78/1160 64	78/1160 65	78/1160 66	78/1162 67	78/1162 78	78/1162 79	78/1163 10	78/1163 31	78/1165 57
LIVER, CONSENSUS		N																	N
Cholangiocarcinoma																			
Hepatocellular Adenoma																			
Hepatocellular Carcinoma																			
Histiocytic Sarcoma																			
Lymphoma, Malignant			P																
Angiectasis														1					
Bile Duct Hyperplasia	3			2	2	3	2	3	4	2	3	3	1	2	2	4	1	2	2	
Cholangiofibrosis																			
Cystic Bile Duct																			
Degeneration, Cystic			1							1	1			1					
Dilated Bile Ducts, Focal																			
Fatty Change, Centrilobular																			
Fatty Change, Diffuse						2													
Fatty Change, Focal																			
Fatty Change, Periportal																			
Fibrosis, Centrilobular																			
Fibrosis, Periportal	2					1			3							2		1	
Focus/Foci, Basophilic																			
Focus/Foci, Clear Cell	2				1			2		2					1				
Focus/Foci, Eosinophilic							1	2		2	2		3	2			1		2	
Focus/Foci, Mixed Cell										1									
Hepatitis, Necrotizing, Multifocal																			
Hepatitis, Suppurative, Multifocal							1												
Hepatocellular Hyperplasia																			
Hepatocytomegaly, Centrilobular																			
Hyperplasia, Focal																			
Necrosis, Centrilobular																			
Necrosis, Focal																			
Necrosis, Multifocal																			
Oval Cell Proliferation																			
Pericholangitis, Granulomatous									5										
Pigment Deposition																			

EPL | Experimental Pathology Laboratories, Inc.

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP CO

ANIMAL	78/16/59	78/16/64	78/16/69	78/16/70	78/17/78	78/18/80	78/18/83	78/18/86	78/18/87	78/18/88	78/18/89	78/17/06	78/17/07	78/17/12	78/17/14	78/17/15	78/17/16	78/17/22	78/17/37	78/17/40
LIVER, CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma	P			P																P
Hepatocellular Carcinoma																				
Histiocytic Sarcoma																				
Lymphoma, Malignant																		P		
Angiectasis																				3
Bile Duct Hyperplasia	2	2	2	4	2	2		3	2	2	2	3	2	3	2	2	2	2	2	3
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic										1									2	
Dilated Bile Ducts, Focal										1			2							5
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																			1	
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal			2	2				2		1	2	2	2	2			1			
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell					2				1											
Focus/Foci, Eosinophilic	3				1	3			2					1	2	2	1	2	2	4
Focus/Foci, Mixed Cell															2					2
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal							1													
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

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Experimental Pathology Laboratories, Inc.

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP
CO

ANIMAL	78/1175 0	78/1176 8	78/1179 3	78/1182 4	78/1184 2	78/1185 7	78/1186 5	78/1187 5	78/1188 5	78/1190 2	78/1190 5
LIVER, CONSENSUS											
Cholangiocarcinoma											
Hepatocellular Adenoma											P
Hepatocellular Carcinoma											
Histiocytic Sarcoma											
Lymphoma, Malignant											
Angiectasis											
Bile Duct Hyperplasia	3	3	2	3	2	4	3	3	3	3	3
Cholangiofibrosis											
Cystic Bile Duct											
Degeneration, Cystic											1
Dilated Bile Ducts, Focal											
Fatty Change, Centrilobular											
Fatty Change, Diffuse											
Fatty Change, Focal											
Fatty Change, Periportal											
Fibrosis, Centrilobular											
Fibrosis, Periportal		2		2	1	2					
Focus/Foci, Basophilic											
Focus/Foci, Clear Cell											
Focus/Foci, Eosinophilic	1	2	3	2	4	3	3	3	3	3	2
Focus/Foci, Mixed Cell						2				3	
Hepatitis, Necrotizing, Multifocal											
Hepatitis, Suppurative, Multifocal											
Hepatocellular Hyperplasia											
Hepatocytomegaly, Centrilobular											
Hyperplasia, Focal											
Necrosis, Centrilobular											
Necrosis, Focal					1						
Necrosis, Multifocal											
Oval Cell Proliferation											
Pericholangitis, Granulomatous											
Pigment Deposition											

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP 30

ANIMAL	78/63	77/62	77/71	77/92	77/97	78/105	78/117	78/118	78/128	78/129	78/153	78/220	78/236	78/237	78/322	78/353	78/555	78/563	78/697	78/804
LIVER, CONSENSUS			A	X												X	X			
Cholangiocarcinoma																				
Hepatocellular Adenoma											P									
Hepatocellular Carcinoma																				
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia		1				3	2		1		2		2	3	1			3	3	4
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																		1		
Dilated Bile Ducts, Focal																				
Fatty Change, Centrilobular																		1		
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal																				3
Focus/Foci, Basophilic										2								1		
Focus/Foci, Clear Cell								2	1					2				1		
Focus/Foci, Eosinophilic	3				1			2		3		2	2		4			3	4	2
Focus/Foci, Mixed Cell	3					2				2	2			2						
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular						1														
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal								2												1
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP 30

ANIMAL	78/858	78/946	78/947	78/961	77/1160	77/1164	77/1183	77/1432	77/1686	77/1780	77/1915	78/1100	78/1166	78/1254	78/1304	78/1318	78/1326	78/1347	78/1356	78/1434
LIVER, CONSENSUS								X			N									N
Cholangiocarcinoma																				
Hepatocellular Adenoma																			P	
Hepatocellular Carcinoma																				
Histiocytic Sarcoma																				
Lymphoma, Malignant										P										
Angiectasis																			4	
Bile Duct Hyperplasia	3	2	3	1	2		1		1	3		2	3	2	3	3	3	2	4	
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal															1					
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal									1						1				2	
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell				1														3	2	
Focus/Foci, Eosinophilic		1		1		2						1		3			2		5	
Focus/Foci, Mixed Cell														2			2	2		
Hepatitis, Necrotizing, Multifocal									2											
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																			P	
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular					3															
Necrosis, Focal							1													
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

EPL
Experimental Pathology Laboratories, Inc.

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP 30

ANIMAL	78/14/60	78/14/71	78/14/78	78/15/27	78/15/54	78/15/57	78/15/80	78/15/88	78/15/89	78/15/98	78/16/09	78/16/10	78/16/11	78/16/38	78/16/60	78/16/62	78/16/65	78/16/67	78/16/78	78/16/74
LIVER, CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma												P		P	P					
Hepatocellular Carcinoma																				
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia	1	4	2	3	4	3	2	3	2	3	2	3	3		3	1	2		2	2
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal					1												1			
Fatty Change, Centrilobular			1																	
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fatty Change, Periportal							2													
Fibrosis, Centrilobular																				
Fibrosis, Periportal	1	2																	2	
Focus/Foci, Basophilic								2												
Focus/Foci, Clear Cell												2		2		2		2		
Focus/Foci, Eosinophilic		2	2	2	2		3		2	2	3	5	2	3	3	2		3	2	2
Focus/Foci, Mixed Cell					2		2	2											3	
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular		1																		
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

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m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

- Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP 30

ANIMAL	78/16 75	78/16 79	78/16 81	78/16 82	78/17 03	78/17 04	78/17 05	78/17 08	78/17 38	78/17 39	78/17 51	78/17 53	78/17 54	78/17 55	78/17 56	78/17 57	78/17 59	78/17 60	78/17 64	78/17 65
LIVER, CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma	P							P												
Hepatocellular Carcinoma																			P	
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis		3								2										
Bile Duct Hyperplasia	3	2	2	3	2	2	1	2	2	3	3	2	2	3	3	2	2	2	3	
Cholangiofibrosis																				
Cystic Bile Duct														3						
Degeneration, Cystic																				
Dilated Bile Ducts, Focal					1															
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal	2						1						2	2	2					
Focus/Foci, Basophilic										3	2									
Focus/Foci, Clear Cell		2		1			3								2	1			1	
Focus/Foci, Eosinophilic	2	2		3	3	4	3		2	3	3	3	2	2	2	2	3	2		3
Focus/Foci, Mixed Cell		3			2	2	2				3	3		3	3					
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

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HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

	GROUP 30																			
ANIMAL	78/11770	78/11771	78/11774	78/11775	78/11779	78/11780	78/11781	78/11782	78/11789	78/11790	78/11796	78/11797	78/11799	78/11801	78/11802	78/11807	78/11818	78/11819	78/11820	78/11822
LIVER, CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma			P			P	P								P					
Hepatocellular Carcinoma																				
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis							2													
Bile Duct Hyperplasia	3	2	2	2	2	3	3		2	3		2	1	3	2	3	2	1	1	2
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic														1			2			
Dilated Bile Ducts, Focal							2								1					
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal					1	2							1		1					
Focus/Foci, Basophilic															2					
Focus/Foci, Clear Cell	2	3		2		2		1			1					3		2		
Focus/Foci, Eosinophilic	2	1	3	4	2	3	3	2	3	3	2			2	3	2	4	5	3	3
Focus/Foci, Mixed Cell		2		2	2	2			3	4		3		2	3			3	3	2
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal																				
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

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HISTOPATHOLOGY INCIDENCE TABLE

GROUP 30

Pos Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

ANIMAL	78/118225	78/118266	78/118277	78/118288	78/118299	78/118355	78/118388	78/118399	78/118400	78/118411	78/118443	78/118455	78/118460	78/118622	78/118633	78/118766	78/118870	78/118872	78/118878	78/118879
LIVER, CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma		P													P					
Hepatocellular Carcinoma																				
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis						1														
Bile Duct Hyperplasia	4	3	4	3	2	3		3	3	3	2	3	2	3	2	3	3	2	3	2
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic				1						2										
Dilated Bile Ducts, Focal										1										
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal	2										1					2				
Focus/Foci, Basophilic			2		1		2							3			2			
Focus/Foci, Clear Cell					1	3	1	2	3			2	2		3				3	
Focus/Foci, Eosinophilic	2	1	2	3	3	3	3	3	3	3	2	4	5	3	3	3	4	2	2	3
Focus/Foci, Mixed Cell	4			3		3			3	3		3	2		2	2	3		2	3
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal												1								
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

EPL
Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant .
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCE Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP
30

ANIMAL	78/118866	78/118867	78/119001	78/119003	78/119006	78/119007	78/119015	78/119016	78/119017	78/119018
LIVER, CONSENSUS										
Cholangiocarcinoma										
Hepatocellular Adenoma			P							
Hepatocellular Carcinoma				P						
Histiocytic Sarcoma										
Lymphoma, Malignant										
Angiectasis			3							
Bile Duct Hyperplasia	2	2	3	2		4	2	3	3	3
Cholangiofibrosis										
Cystic Bile Duct										3
Degeneration, Cystic										
Dilated Bile Ducts, Focal	1									
Fatty Change, Centrilobular										
Fatty Change, Diffuse										
Fatty Change, Focal										
Fatty Change, Periportal										
Fibrosis, Centrilobular										
Fibrosis, Periportal										
Focus/Foci, Basophilic			3	1		2		2		
Focus/Foci, Clear Cell	2		2		2		3		1	
Focus/Foci, Eosinophilic	2	2	4	3	2	3	4	4	3	2
Focus/Foci, Mixed Cell	2	2	2	2	2					
Hepatitis, Necrotizing, Multifocal										
Hepatitis, Suppurative, Multifocal										
Hepatocellular Hyperplasia										
Hepatocytomegaly, Centrilobular										
Hyperplasia, Focal										
Necrosis, Centrilobular										
Necrosis, Focal										
Necrosis, Multifocal										
Oval Cell Proliferation										
Pericholangitis, Granulomatous										
Pigment Deposition										

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

GROUP 60

Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

ANIMAL	78/70	77/62	77/79	77/82	78/11	78/18	78/24	78/50	78/55	78/69	78/76	78/85	78/91	78/94	77/19	77/11	78/00	78/02	78/04	78/09	78/10	78/08
LIVER, CONSENSUS	X					N																
Cholangiocarcinoma																						
Hepatocellular Adenoma		P			P		P	P	P	P	P	P	P	P		P		P		P		P
Hepatocellular Carcinoma													P								P	
Histiocytic Sarcoma			P																			
Lymphoma, Malignant				P																		
Angiectasis																						
Bile Duct Hyperplasia		3		2	3		2	2	2	3	2	3	2	2			3	2				2
Cholangiofibrosis																						
Cystic Bile Duct																						
Degeneration, Cystic												1										
Dilated Bile Ducts, Focal		2										1										
Fatty Change, Centrilobular																						
Fatty Change, Diffuse																						
Fatty Change, Focal												2										
Fatty Change, Periportal									2													
Fibrosis, Centrilobular																						
Fibrosis, Periportal							2				2		3									
Focus/Foci, Basophilic																1						
Focus/Foci, Clear Cell								2			3	2			3	3						2
Focus/Foci, Eosinophilic		2					4	3	4	2	5		3			2	2	4				3
Focus/Foci, Mixed Cell							3			2	3			3				2				
Hepatitis, Necrotizing, Multifocal																						
Hepatitis, Suppurative, Multifocal										2												
Hepatocellular Hyperplasia																						
Hepatocytomegaly, Centrilobular																	2					
Hyperplasia, Focal																						
Necrosis, Centrilobular																						
Necrosis, Focal		3			4				2			2								5		
Necrosis, Multifocal																						
Oval Cell Proliferation																						
Pericholangitis, Granulomatous																						
Pigment Deposition																						

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP 60

ANIMAL	78/11/10 86	78/11/11 64	78/11/12 18	78/11/13 23	78/11/33 25	78/11/33 42	78/11/33 45	78/11/34 46	78/11/34 55	78/11/34 87	78/11/34 96	78/11/44 66	78/11/44 41	78/11/44 12	78/11/45 22	78/11/45 54	78/11/47 40	78/11/47 79	78/11/48 33	78/11/49 66
LIVER, CONSENSUS																	A			A
Cholangiocarcinoma																				
Hepatocellular Adenoma			P		P	P	P	P		P		P	P			P	P	P		
Hepatocellular Carcinoma	P		P	P					P	P			P		P	P			P	
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis								4												
Bile Duct Hyperplasia	2	2	3	2	2	3	2	2	3	3	3	3	2		2	3		3		
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal										2										
Fatty Change, Centrilobular																				
Fatty Change, Diffuse											3									
Fatty Change, Focal																				
Fatty Change, Periportal		2																		
Fibrosis, Centrilobular																				
Fibrosis, Periportal											3	2			2					
Focus/Foci, Basophilic					3							2								
Focus/Foci, Clear Cell					4			2						3						
Focus/Foci, Eosinophilic	3	3	3	2	3	4	3	3	4	3		3	4		2	4		4		
Focus/Foci, Mixed Cell		2						2						3						
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia											P									
Hepatocytomegaly, Centrilobular							2													
Hyperplasia, Focal											3									
Necrosis, Centrilobular											3									
Necrosis, Focal									3											
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

GROUP 60

Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

ANIMAL	78/15/25	78/15/26	78/15/28	78/15/30	78/15/35	78/15/36	78/15/38	78/15/39	78/15/44	78/15/45	78/15/46	78/15/47	78/16/07	78/16/08	78/16/34	78/16/35	78/16/36	78/16/37	78/16/39	78/16/58
LIVER, CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma		P	P	P		P	P	P	P	P	P	P	P	P		P	P	P	P	P
Hepatocellular Carcinoma				P	P	P				P					P	P			P	
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis																				
Bile Duct Hyperplasia	4	3	3	2	3	3	2	4	2	3	3		3	2	3	3	2	3	2	2
Cholangiofibrosis																				
Cystic Bile Duct				3				3												
Degeneration, Cystic				1																
Dilated Bile Ducts, Focal																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																		2		
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal				2	2			3							2					2
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell									2		2					1			2	
Focus/Foci, Eosinophilic		5	5			4	3	3	4	4	5	5	5	5	3	4	3	3	4	4
Focus/Foci, Mixed Cell									3						3					
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal			2	3	5															
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition											1									

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=mori[illegible] anc./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP 60

ANIMAL	781/1 66661	781/1 66663	781/1 66666	781/1 66671	781/1 66676	781/1 66677	781/1 66684	781/1 67709	781/1 67710	781/1 67711	781/1 67713	781/1 67717	781/1 67736	781/1 67741	781/1 67742	781/1 67743	781/1 67744	781/1 67745	781/1 67752	781/1 67758
LIVER CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma	P		P	P	P		P							P		P			P	P
Hepatocellular Carcinoma		P		P	P	P		P	P	P	P	P	P	P		P	P	P	P	
Histiocytic Sarcoma											P									
Lymphoma, Malignant																				
Angiectasis					3									3						2
Bile Duct Hyperplasia	2	2	3	3	2			2			3	3	2		3	3	3	3	3	2
Cholangiofibrosis												5								
Cystic Bile Duct																				
Degeneration, Cystic						1	2													
Dilated Bile Ducts, Focal																			3	
Fatty Change, Centrilobular									1											
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal	1															2				
Focus/Foci, Basophilic														3						
Focus/Foci, Clear Cell																				
Focus/Foci, Eosinophilic	4	4	4	3	5			3	3	5	3	3		5		4	4	2	4	4
Focus/Foci, Mixed Cell		3									3				5	4		3		
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal			4		3		2													
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition													2							

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP 60

ANIMAL	78/11761	78/11762	78/11763	78/11769	78/11772	78/11773	78/11776	78/11777	78/11778	78/11788	78/11791	78/11792	78/11794	78/11798	78/11800	78/11816	78/11821	78/11823	78/11830	78/11833
LIVER, CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma	P		P		P	P	P		P	P	P	P		P		P	P	P	P	P
Hepatocellular Carcinoma		P		P			P	P	P	P	P	P	P		P			P		P
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis																				3
Bile Duct Hyperplasia	2	1	2	2	2		3	3	2	3	2	3		3	2	3	1	2		3
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic																				
Dilated Bile Ducts, Focal																				
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal									2											
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell					2	4	2					2							3	
Focus/Foci, Eosinophilic	5	3	3	2	4	4		3	4	3	4	3	3	3	4		3	3	3	3
Focus/Foci, Mixed Cell					3					3			2			5			3	
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular																				
Necrosis, Focal				4														4	2	
Necrosis, Multifocal																				
Oval Cell Proliferation																				
Pericholangitis, Granulomatous																				
Pigment Deposition																				

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

PCB Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

GROUP 60

ANIMAL	78/11/83 34	78/11/83 36	78/11/83 37	78/11/84 44	78/11/85 44	78/11/85 55	78/11/85 56	78/11/85 58	78/11/85 59	78/11/86 61	78/11/86 67	78/11/86 68	78/11/86 69	78/11/87 71	78/11/87 73	78/11/87 74	78/11/87 77	78/11/90 70	78/11/90 04	78/11/91 19
LIVER, CONSENSUS																				
Cholangiocarcinoma																				
Hepatocellular Adenoma					P	P	P		P	P	P	P	P	P	P	P	P		P	P
Hepatocellular Carcinoma	P	P	P	P		P	P	P	P	P		P	P	P	P	P	P	P		P
Histiocytic Sarcoma																				
Lymphoma, Malignant																				
Angiectasis	3					3														
Bile Duct Hyperplasia			4		3	2	3		3	3	2	2	3	3	2	2	3	2	3	3
Cholangiofibrosis																				
Cystic Bile Duct																				
Degeneration, Cystic		2						2												
Dilated Bile Ducts, Focal							2	2												
Fatty Change, Centrilobular																				
Fatty Change, Diffuse																				
Fatty Change, Focal																				
Fatty Change, Periportal																				
Fibrosis, Centrilobular																				
Fibrosis, Periportal																				
Focus/Foci, Basophilic																				
Focus/Foci, Clear Cell	2				2				2	3	4								2	2
Focus/Foci, Eosinophilic	2		4		5	4	5	5	4	3	5	5	4	5	4	3	2	3	4	4
Focus/Foci, Mixed Cell						3													4	3
Hepatitis, Necrotizing, Multifocal																				
Hepatitis, Suppurative, Multifocal																				
Hepatocellular Hyperplasia																				
Hepatocytomegaly, Centrilobular																				
Hyperplasia, Focal																				
Necrosis, Centrilobular												2								
Necrosis, Focal	3	3					3	3												
Necrosis, Multifocal																				
Oval Cell Proliferation																				2
Pericholangitis, Granulomatous																				
Pigment Deposition					2															2

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

GROUP
60

Liver Reassessment
GSF/CLOPHEN30-60 Sacrifice
Rat

ANIMAL	78/119201	78/119202	78/119203	78/119204	78/119205	78/119206
LIVER, CONSENSUS						
Cholangiocarcinoma						
Hepatocellular Adenoma	P	P	P	P	P	P
Hepatocellular Carcinoma	P	P	P	P		P
Histiocytic Sarcoma						
Lymphoma, Malignant						
Angiectasis						
Bile Duct Hyperplasia	2	3	3	2	2	2
Cholangiofibrosis						
Cystic Bile Duct						
Degeneration, Cystic						
Dilated Bile Ducts, Focal			3			
Fatty Change, Centrilobular						
Fatty Change, Diffuse						
Fatty Change, Focal						
Fatty Change, Periportal						
Fibrosis, Centrilobular						
Fibrosis, Periportal						1
Focus/Foci, Basophilic						
Focus/Foci, Clear Cell	2			3	3	
Focus/Foci, Eosinophilic	3	4	4	5	4	3
Focus/Foci, Mixed Cell	3		3	3	3	3
Hepatitis, Necrotizing, Multifocal						
Hepatitis, Suppurative, Multifocal						
Hepatocellular Hyperplasia						
Hepatocytomegaly, Centrilobular						
Hyperplasia, Focal						
Necrosis, Centrilobular						
Necrosis, Focal		2				3
Necrosis, Multifocal						
Oval Cell Proliferation						
Pericholangitis, Granulomatous						
Pigment Deposition						

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=moribund sac./death

REFERENCES

Induction of Liver Tumors in Sherman Strain Female Rats by Polychlorinated Biphenyl Aroclor 1260[1, 2]

Renate D. Kimbrough,[3] Robert A. Squire,[4] Ralph E. Linder,[5] John D. Strandberg,[6] Richard J. Montali,[6] *and* Virlyn W. Burse[3, 7]

SUMMARY—Sherman strain female rats (200) were fed 100 ppm of a polychlorinated biphenyl (Aroclor 1260) for approximately 21 months, and 200 female rats were kept as controls. The rats were killed when 23 months old. Twenty-six of 184 experimental animals and 1 of 173 controls had hepatocellular carcinomas. None of the controls but 146 of 184 experimental rats had neoplastic nodules in their livers, and areas of hepatocellular alteration were noted in 28 of 173 controls and 182 of 184 experimental animals. Thus the polychlorinated biphenyl Aroclor 1260, when fed in the diet, had a hepatocarcinogenic effect in these rats. The incidence of tumors in other organs did not differ appreciably between the experimental and control groups.—J Natl Cancer Inst 55: 1453–1459, 1975.

Polychlorinated biphenyls (PCB's) have been used over the past 44 years as transformer, capacitor, and cooling fluids in various systems (*1*). Since they are excellent dielectrics and flame retardants, many new industrial uses have been found for them, particularly in the past two decades. These compounds are persistent as environmental contaminants and were first established as pollutants in 1966 (*2*). The toxicity of these and related compounds has recently been reviewed (*3*).

In a preliminary feeding study in which the toxicities two PCB mixtures (Aroclor 1254 and Aroclor 1260) re compared (*4*), a bladder tumor was found in 1 of 10 female rats fed 100 ppm Aroclor 1260 in their diet. This bladder tumor was tentatively classified at the time as a poorly differentiated epidermoid carcinoma. Severe autolysis hampered the microscopic examination (Kimbrough RD: Unpublished observation). To establish whether this tumor had developed spontaneously or was induced by the PCB, 200 female rats were fed 100 ppm Aroclor 1260. Another group of 200 females were the controls. This paper reports the results of this study.

MATERIALS AND METHODS

Four hundred weanling Sherman strain COBS[8] female rats 21–26 days old and weighing 48–97 g were distributed into two groups of 200 animals each according to a table of random numbers. Ten animals were housed per cage in conventional humidity, light, and temperature-controlled surroundings. Two hundred rats were fed plain ground Purina Laboratory Chow and 200 were fed the same diet containing 100 ppm Aroclor 1260 (lot No. AK-3; Monsanto Industrial Chemical Co., St. Louis, Mo.). For incorporation of Aroclor 1260 into the diet, 5.2 g was dissolved in ethyl ether; this was added to 100 g cornstarch, and the ether was allowed to evaporate. The PCB cornstarch mixture was blended with increasing amounts of ground chow. The diets were prepared fresh every 10–14 days. Random samples from the final mixes and samples of control chow were taken at egular intervals to determine PCB levels, at first biekly and later bimonthly.

For determination of the PCB levels, the samples were extracted for 2 hours on an automatic shaker with a 3:1 mixture of hexane and isopropanol (*5*). After filtration of the extract through glass wool, the extracts were washed with a 2% sodium chloride solution and dried with sodium sulfate. Samples were eluted through Florisil (*6*). The 6% fraction was collected and eluted through the silicic acid–celite column of Armour and Burke (*7*). Samples were analyzed by electron-capture, gas–liquid chromatography, and/or Coulson Conductometric gas–liquid chromatography.

During the first year of the study, three batches of food, both when first received and after having been in the food cups for a week, were analyzed for aflatoxins (*8*).

The combined body weight of rats in each cage was recorded weekly until the rats were 6 months old, biweekly until 12 months, and monthly thereafter. Individual weights were recorded only at the onset of the experiment and at death or when the animals were killed. The rats were observed briefly each day, and those exhibiting debilitating signs or large tumors were removed from the group cage and housed individually. At each weighing the animals were examined individually and abnormalities were noted. Food consumption was measured on all rats during the first 2 weeks of the experiment, and then during weeks 5, 8, 11, and 20, and every 12 weeks thereafter. The dietary exposure of the experimental group to Aroclor 1260 was discontinued 6 weeks before they were killed. Autopsies were performed on all that died. When the animals were 23 months old, they were anesthetized and their venae cavae were severed. Tissues were fixed in 10% buffered formalin and stained with hematoxylin and eosin. Selected tissue sections were stained with periodic acid-Schiff, Wilder's reticulum, azure eosin, and Masson's trichrome. In addition to tissue masses, the following organs were studied microscopically: brain, pituitary, thyroid, parathyroid, tra-

[1] Received May 20, 1975; accepted July 24, 1975.

[2] Supported in part by Public Health Service contracts N01 CP43300 and N01 CP43288 from the Division of Cancer Cause and Prevention, National Cancer Institute.

[3] Toxicology Branch, Center for Disease Control, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Ga. 30333.

[4] National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014.

[5] Environmental Protection Agency (EPA), Research Triangle Park, N. C. 27711.

[6] The Johns Hopkins University, School of Medicine, Baltimore, Md., 21205.

[7] We thank Mr. Martin Goldstein, Federal Drug Administration (FDA), New Orleans District, for the feed analysis for aflatoxins and Mr. Sol Cohen, FDA, Atlanta District, for helpful suggestions. Mr. Richard Moore (EPA) assisted with animal care and autopsies.

[8] The Sherman rats are descendants of the Sherman strain developed at Columbia University from the Osborne Mendel strain. They have been randomly bred in our colony at the Center for Disease Control since 1950. They were cesarean obtained and barrier sustained (COBS) in 1966. In conventional rats, the incidence of murine pneumonia was high. Since they have been COBS, this is no longer a problem.

chea, esophagus, lung, stomach, kidney, heart, liver, spleen, adrenal, ovary, uterus, and urinary bladder. Student's *t*-test was used for comparison of mean body weights and mean weight gain.

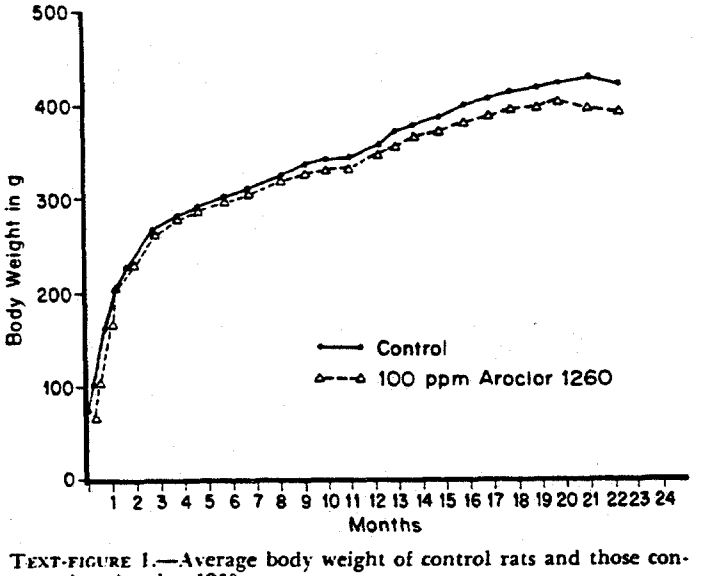
RESULTS

The PCB concentrations in the experimental diet ranged usually from 70 to 107 ppm. Occasionally, lower values were found at the beginning of the study. The occurrence of low PCB concentrations in the experimental diet was later resolved by improvement of the extraction method. PCB's in the control diets were less than 0.1 ppm and usually below the limit of detection. Aflatoxins were not detected in either diet. The sensitivity levels were 2.5 ppb aflatoxins B_1 and G_1 and 0.1 ppb aflatoxins B_2 and G_2. A study in which aflatoxin B_1 was added to a portion of the feed showed 94% recovery.

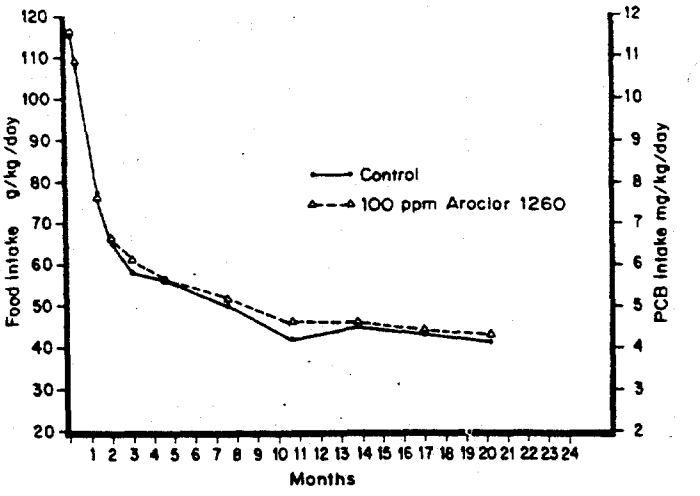
No definite dose-related signs of toxicity were observed in the test animals. Comparative curves of body-weight gain and food consumption on the basis of body weight, as well as PCB intake of the test group, are given in text-figures 1 and 2. A slight decline in the rate of weight gain in the test group compared to that in the control group began about 3 months after onset of the experiment. Mean final body weights were 420 g (SD = 72 g, SE = 5.4 g) for the control group and 392 g (SD = 62 g, SE = 4.6 g) in the test group; the difference was statistically significant ($P < 0.001$). Food consumption (text-fig. 2) was comparable in both groups. Mean weight gain was 350 g (SD = 70 g, SE = 5.3 g) and 323 g (SD = 60 g, SE = 4.5 g) for the control and test groups, respectively; the difference in weight gain was also statistically significant ($P < 0.001$). PCB intake declined from 11.6 mg/kg/day during the first week of exposure to 6.1 mg/kg/day at 3 months of exposure and to 4.3 mg/kg/day at 20 months (text-fig. 2).

Pathologic Findings

Control rats (173) and experimental animals (184) were examined grossly and microscopically. The experimental group included 5 rats that were killed 1–2 months before the final kill. The remaining animals



TEXT-FIGURE 1.—Average body weight of control rats and those consuming Aroclor 1260.



TEXT-FIGURE 2.—Food intake of control and experimental rats and consumption of Aroclor 1260 by experimental rats.

were not included because of improper tissue fixation or because they died early in the experiment.

A consistent difference in the appearance of the livers was observed between the experimental and control groups. Almost all (176/184) livers of the experimental animals had from a few to multiple elevated tan nodules on the surfaces; additional nodules were usually seen on sectioning. These nodules varied from 0.1 to several cm in diameter, and in some rats replaced almost the entire liver. In contrast, the liver of only one control showed gross abnormalities and was markedly enlarged, nodular, tan, and firm. In addition, a variety of tumors of other organs was observed in both the experimental and the control groups. The incidence and type of tumors are given in table 1.

Histologic examination showed that 26 experimental animals and 1 control with enlarged, nodular livers had hepatocellular carcinomas. The additional 144 experimental rats with gross liver nodularity had hepatocellular nodules characteristic of neoplastic nodules [synonym, "hyperplastic nodules" (*9*)]. A recent workshop sponsored by The National Cancer Institute[9] recommended the term "neoplastic nodules" for these lesions as a more accurate indication of their biologic significance. No nodules were in control animals.

The hepatocellular carcinomas were well-differentiated trabecular types (figs. 1–3), except for three in the experimental animals which had a glandular, papillary pattern (fig. 4). The trabecular tumors showed severe disruption of normal liver architecture and were usually easily recognized at low magnification. Liver plates, two or more cells thick in some areas, were arranged in haphazard linear, branching, or pseudoglandular patterns. Different patterns were usually present in the same tumor. Blunt-ended plates, sinusoidal ectasia, and congestion were frequent. The hepatocytes varied from a normal appearance to enlarged, acidophilic, or diffusely basophilic cells with large, hyperchromatic nuclei and prominent nucleoli. The cytoplasm often contained eosinophilic inclusions within vacuoles. Mitotic figures were sometimes present. Foci of coagulative necrosis were occasionally observed in cancerous areas, but there

[9] Rat Liver Tumor Workshop at Silver Spring, Md., Dec. 11–13, 1974; sponsored by Carcinogenesis, Division of Cancer Cause and Prevention, National Cancer Institute.

TABLE 1.—*Incidence and type of liver lesions and tumors of other organs examined histologically*

Organ or tissue	Type of lesion	Incidence	
		Controls	Experimental
Liver	Hepatocellular carcinoma	1/173	26/184
	Neoplastic nodules	0/173	144/184
	Foci or areas of cytoplasmic alteration	28/173	182/184
Thyroid gland	Parafollicular cell tumor	37/160	18/166
Adrenal gland	Pheochromocytoma	1/173	1/167
Pituitary gland	Chromophobe adenoma	41/153	28/139
	Carcinoma	0/153	1/139
Uterus	Endometrial polyp	18/149	25/163
	Adenocarcinoma	0/149	2/163
	Sarcoma of endometrial stroma	3/149	7/163
Urinary bladder	Transitional cell papilloma	1/167	0/169
Mammary gland	Fibroadenoma	17/173[a]	13/184[a]
	Adenocarcinoma	5/173[a]	1/184[a]
Salivary gland	Fibrosarcoma	1/173[a]	0/184[a]
Lung	Adenoma	2/173	2/184
Adipose tissue	Lipoma	0/173[a]	2/184[a]
Brain	Glioma	0/173	2/184
Ovary	Granulosa theca cell tumor	5/149	0/163
	Papillary adenoma	1/149	2/163
Hematopoietic system	Granulocytic leukemia	1/173	0/184
	Lymphoma	0/173	2/184
Kidney	Hemangioma	0/173	1/184
Thymus	Thymoma	1/173[a]	0/184[a]
Parathyroid gland	Adenoma	0/173	2/184
Skin	Fibroma	0/173[a]	1/184[a]

[a] Incidence based on gross detection with microscopic confirmation.

was no fibrosis or other evidence of chronic degenerative changes. Periodic acid-Schiff (PAS) without diastase stained carcinomas less intensely than uninvolved liver, which suggested a decrease in glycogen. Most carcinomas were more basophilic than the normal liver with azure eosin stain. No definite intravascular invasion or metastases were found.

Neoplastic nodules were generally spherical and well demarcated, and occupied areas equal to those of several liver lobules (fig. 5). The cells in these nodules were generally enlarged, and the cytoplasm was either ground-glass-appearing, diffusely basophilic, or clear. Enlarged hyperchromatic nuclei, double nuclei, and mitotic figures were often present. The cytoplasm frequently contained inclusions similar to those in the carcinomas, except they were larger and appeared as whorled, concentric lamellae. In previous studies (*3*) these formations were shown by electron microscopy to represent aggregates of smooth endoplasmic reticulum. The normal liver architecture was absent within nodules, and cells were in sheets or irregular plates. Portal areas and central veins were absent and sinusoids were dilated in some areas. At the periphery of the nodules, the surrounding liver plates were tangentially arranged and narrowed, due to compression (figs. 6, 7). Nodules varied in PAS positivity, but always differed from surrounding liver. Most nodules were more eosinophilic, and a few were more basophilic than the normal liver with azure eosin stain.

In 182 treated and 28 control animals, there were also foci or areas of hepatocytes with altered cytoplasm (figs. 8–10). In controls, these were mostly collections of cells with water-clear cytoplasm. In treated animals, most affected cells were enlarged and had eosinophilic, ground-glass-appearing cytoplasm or were diffusely basophilic and smaller than normal cells. In basophilic areas, sinusoids were dilated and liver plates somewhat tortuous. In general, the cells in these areas were like those in neoplastic nodules, but there were no architectural alterations, and plates of involved liver cells merged with the surrounding liver.

No unusual features were noted in tumors of other organs, and there were no apparent differences in incidence between experimental and control animals. Parafollicular thyroid tumors varied from small circumscribed nodules of pale, oval-to-spindle cells to masses that obliterated the gland and invaded the capsule. They were similar to those described by Boorman et al. (*10*). Other frequent tumors included pituitary chromophobe adenomas, mammary fibroadenomas, and endometrial polyps. Endometrial stromal sarcomas were also present in 10 animals.

The only bladder tumor observed was in a control animal. Therefore, the occurrence of the bladder tumor in the previous experiment was apparently unrelated to the ingestion of Aroclor 1260.

A few rats in the experimental group also showed areas of adenofibrosis (synonym, "cholangiofibrosis") of the liver, a lesion described previously following the administration of Aroclor 1260 (*4*).

DISCUSSION

The livers of treated animals showed neoplastic lesions in 170 of the 184 examined, and in only 1 of 173 controls. Although only 26 of the lesions in treated rats were clearly carcinomas according to traditional histologic criteria, neoplastic nodules are part of the spectrum of response to hepatocarcinogens and must be included in the evaluation of tumorigenesis.

In the past, these liver lesions have been interpreted in various ways, and different names have been used to report them: nodular hyperplasia, hyperplastic nodules (*11*), hepatomas (*12, 13*), and hepatic nodules (*14*). Similar ambiguity exists in the classification of human liver lesions of this type (*15*).

Several studies with known carcinogens have demonstrated the development of liver nodules, indistinguishable from the neoplastic nodules in this study, before the appearance of carcinoma (*11, 16–18*). In a recent review (*9*), the biology and significance of nodules and their relationship to hepatocellular carcinoma were thoroughly discussed. In our study, Aroclor 1260 induced a spectrum of nodules and cancers in treated animals as outlined by the Rat Liver Tumor Workshop.[9]

A few Japanese polychlorinated biphenyls and the American product, Aroclor 1254, have been shown to induce neoplastic liver lesions in rodents. In a study with male BALB/cJ mice, nodules were observed in the liver after 11 months' exposure to Aroclor 1254 (*19*). The Japanese PCB's Kanechlor 400 and 500 have reportedly produced liver tumors in female Donryu rats and male dd mice, respectively (*20, 21*). PCB's have a promoting effect on liver tumor induction by benzene hexachloride (*21*), whereas they protected male Sprague-Dawley rats from the tumor-inducing effect of three hepatocarcinogens: 3′-methyl-4-dimethylaminoazobenzene, *N*-2-fluorenylacetamide, and diethylnitrosamine (*22*). The authors suggested that this protective effect may be due to the induction of microsomal enzymes by PCB's. The animals treated only for 20 weeks probably did not receive

the material long enough for the PCB's to induce tumors.

It is not known whether mixtures of polychlorinated biphenyls that are primarily composed of isomers with four or less chlorines on the ring would also induce liver tumors, or whether only those mixtures containing an appreciable proportion of pentachlorobiphenyl, hexachlorobiphenyl, and heptachlorobiphenyl have this effect. Kanechlor 400 contains 3% dichlorobiphenyl, 32.8% trichlorobiphenyl, 43.8% tetrachlorobiphenyl, 15.8% pentachlorobiphenyl, and 4.6% hexachlorobiphenyl. Kanechlor 500 contains 5% trichlorobiphenyl, 26% tetrachlorobiphenyl, 55% pentachlorobiphenyl, and 13% hexachlorobiphenyl (*21*). Chlorinated dibenzofuran was found in one Kanechlor 400 sample (*23*). The composition of the Aroclor 1260 studied by us is not known. Sissons and Welti (*24*) analyzed Aroclor 1260 manufactured by Monsanto Chemicals Ltd. and concluded that hexachlorobiphenyl and heptachlorobiphenyl were major constituents.

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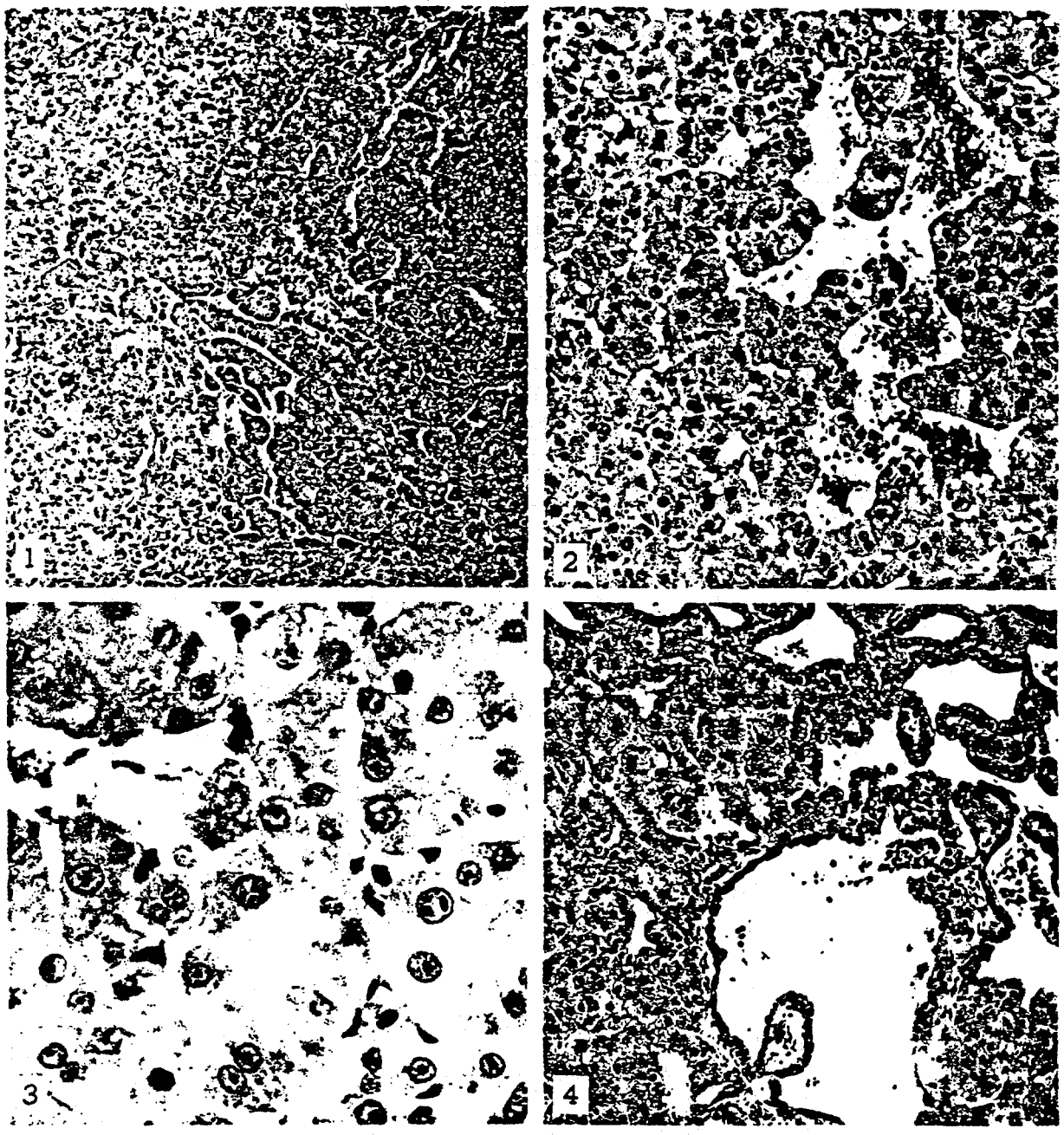

FIGURE 1.—Focus of hepatocellular carcinoma in rat fed Aroclor 1260. Tumor cells are in sheets, irregular nests, and cords. Hematoxylin and eosin (H & E). × 45

FIGURE 2.—Focus of hepatocellular carcinoma in rat fed Aroclor 1260. *Note* plates and nests of cells, two or more cells in thickness, enveloped by lining cells. H & E. × 145

FIGURE 3.—Focus of hepatocellular carcinoma in rat fed Aroclor 1260 showing nests of cells in pseudoacinar patterns. H & E. × 360

FIGURE 4.—Focus of hepatocellular carcinoma with glandular pattern in rat fed Aroclor 1260. H & E. × 105

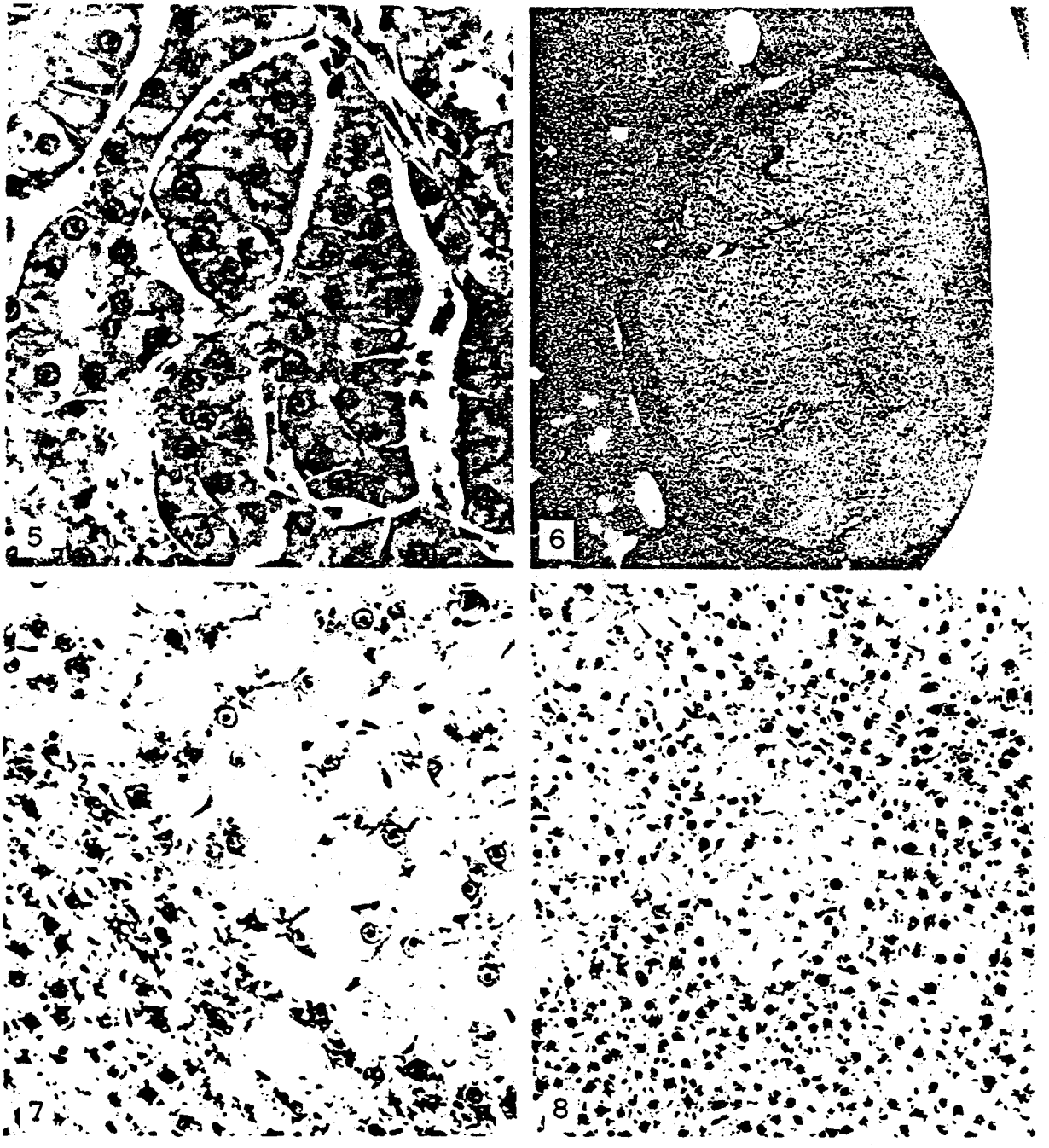

FIGURE 5.—Focus of hepatocellular carcinoma in rat fed Aroclor 1260. *Note* thick cell plates, hyperchromatic nuclei, and prominent nucleoli. H & E. × 360

FIGURE 6.—Neoplastic nodule in rat fed Aroclor 1260. Periphery is sharply demarcated from surrounding parenchyma. H & E. × 10

FIGURE 7.—Edge of neoplastic nodule in figure 6. Surrounding liver plates are compressed and tangentially arranged around nodule. *Note* eosinophilic, lamellar bodies in cytoplasm of nodule cells. H & E. × 330

FIGURE 8.—Area of clear-cell cytoplasmic alteration. H & E. × 185

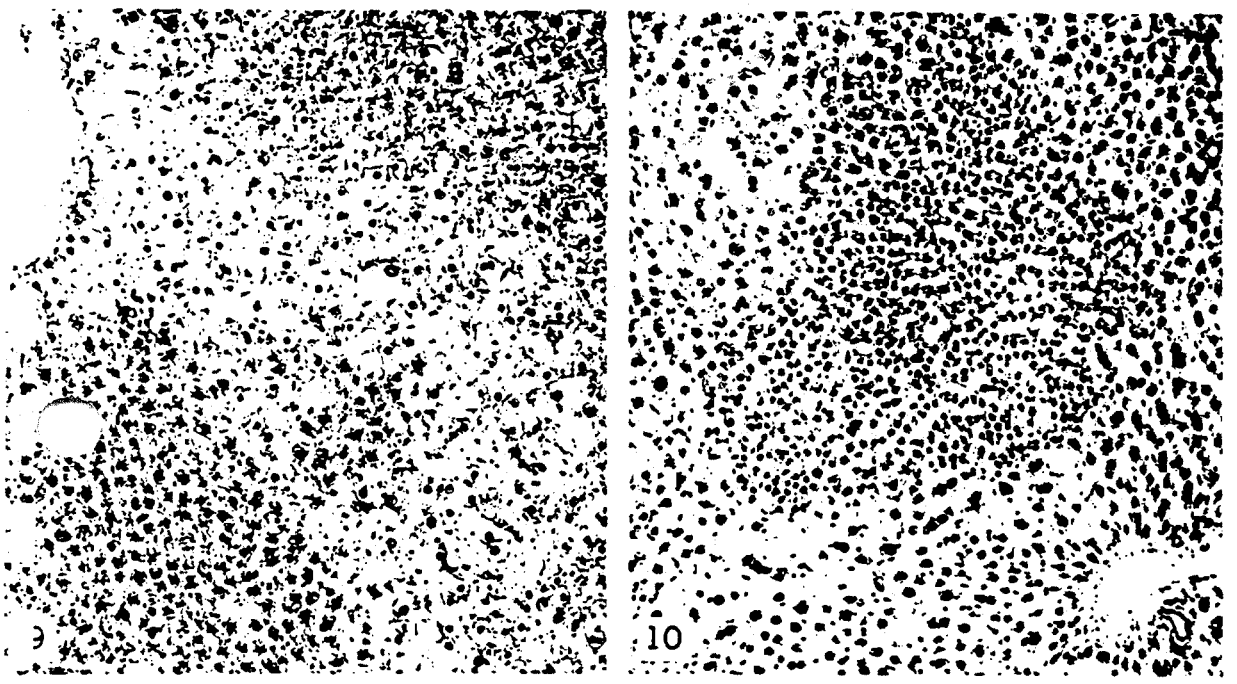

FIGURE 9.—Area of eosinophilic cytoplasmic alteration having the appearance of ground glass. H & E. × 185

FIGURE 10.—Area of basophilic cytoplasmic alteration. H & E. × 105

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THE EFFECT OF POLYCHLORINATED BIPHENYLS ON RAT REPRODUCTION

R. E. LINDER*, T. B. GAINES and R. D. KIMBROUGH

Bio-Effects Branch, Primate and Pesticides Effects Laboratory, Environmental Protection Agency, 4770 Buford Highway, Chamblee, Georgia 30341, USA

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Abstract—Reproduction, pathology and acute toxicity were studied in Sherman rats exposed to the polychlorinated biphenyls, Aroclor 1254 and Aroclor 1260. Rats exposed to Aroclor 1254 at dietary levels of 20 ppm or more had fewer pups per litter than the controls in the F_{1b} and F_2 generations. The 100 ppm exposure level of Aroclor 1254 increased mortality in the F_{1b} offspring and markedly decreased mating performance of the F_{1b} adults. The 500 ppm dietary level of Aroclor 1260 reduced litter size and decreased survival in the F_1 litters. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no effect on reproduction in rats exposed through two generations. Liver weights were increased in 21-day-old F_1 male weanlings at the 1 ppm level of Aroclor 1254 and in either sex of F_1 and F_2 weanlings at 5 ppm or higher levels of both Aroclor 1254 and 1260. Histological changes in the liver and increased liver weights were observed in adult rats exposed to the higher levels. Pregnant rats given Aroclor 1254 at the rate of 100 mg/kg/day on days 7–15 of gestation produced grossly normal litters, but only 30·1% of the pups survived to weaning. Reproduction and pup survival were not affected at dosage rates of 50 mg. Aroclor 1254/kg/day or 100 mg Aroclor 1260/kg/day. Oral LD_{50} values in 3–4-wk-old male rats were 1295 and 1315 mg/kg for Aroclors 1254 and 1260, respectively. The iv LD_{50} for Aroclor 1254 in adult females was 358 mg/kg.

INTRODUCTION

Reproductive defects due to commercial mixtures of polychlorinated biphenyls (PCBs) have been reported in experimental birds (Dahlgren, Linder & Carlson, 1972) and mammals (Ringer, Aulerich & Zabik, 1972). Porphyria (Vos & Koeman, 1970), microsomal-enzyme induction (Street, Urry, Wagstaff & Blau, 1969) and liver damage (Kimbrough, Linder & Gaines, 1972; Miller, 1944) have also been reported in experimental animals exposed to PCB mixtures.

The present investigation was initiated in 1970 to study the effects on reproduction and pathology produced by two American-made PCB mixtures sold under the trade-names Aroclor 1254® and Aroclor 1260®. Aroclor 1254 contains 54% (w/w) chlorine and is composed of 11% tetra-, 49% penta-, 34% hexa- and 6% heptachlorobiphenyls; Aroclor 1260 has 60% (w/w) chlorine, with a composition of 12% penta-, 38% hexa-, 41% septa-, 8% octa- and 1% nonochlorobiphenyls (Thruston, 1971). An almost complete identification of isomers in Aroclor 1254 and Aroclor 1260 has been obtained (Sissons & Welti, 1971). Since 1970, the Monsanto Co., the only US manufacturer of PCBs, has voluntarily reduced sales of Aroclor mixtures and has taken steps to limit their use to closed systems (Monsanto Co., 1971).

The present communication is an account of reproduction studies in rats. Also included are acute toxicity values from preliminary studies and comments on pathology and haematology in animals from the reproduction experiments. A detailed account of the liver pathology of the F_0 rats given 20 ppm and higher dietary levels has been published (Kimbrough *et al.* 1972).

*Present address: United States Environmental Protection Agency, National Environmental Research Center, Research Triangle Park, North Carolina 27711.

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EXPERIMENTAL

Animals and materials. All animals used in the studies were Sherman-strain rats produced under specific-pathogen-free conditions. Aroclor 1254 (Lot AK-38) and Aroclor 1260 (Lot AK-3) were supplied by Monsanto Industrial Chemicals Company, St. Louis, Mo.

Experimental design and conduct

LD_{50} studies. The single-dose oral LD_{50} was determined for Aroclor 1254 and 1260 in weanling male rats (3–4 wk old). The iv LD_{50} was determined in adult females for Aroclor 1254. Between five and eight groups, each of ten rats, were used to obtain each LD_{50} value. The procedure described by Gaines (1969) was followed for oral dosing; Aroclor 1254 or Aroclor 1260 was dissolved in peanut oil and the solution was administered by oral intubation at the rate of 5 ml/kg. For iv dosing, Aroclor 1254 was first dissolved in peanut oil. One part of this solution was added to nine parts of a 1% lecithin–saline suspension and homogenized. This formulation was administered in a single injection via the tail vein at the rate of 5 ml/kg. A control group of five rats received 10% peanut oil in lecithin–saline in the same volume. Survivors were observed for 10–14 days.

Two-generation reproduction studies. For these studies the rats were fed either untreated ground laboratory chow (control) or ground chow fortified with Aroclor 1254 or Aroclor 1260. The Aroclors were dissolved in ether and mixed with cornstarch. The ether was allowed to evaporate and the Aroclor–cornstarch mixture was mixed into increasing amounts of ground chow. Aroclor 1254 was fed at dietary levels of 0 (control), 1, 5, 20 and 100 ppm, while Aroclor 1260 was fed at levels of 0, 5, 20 and 100 ppm. Because exposure schedules had to be staggered, a specific control group was added each time one or more treated groups were started on a dietary regimen. The F_0 rats were started on the diets at 3–4 wk of age and the F_{1b} rats at weaning. Exposure was continuous through mating, gestation and lactation until the rats were killed. Ten males and 20 females were fed at each dietary level in both the F_0 and F_{1b} generations. The F_0 males at each dietary level and half of the females were caged individually. Other F_0 females and all the F_{1b} rats were caged in groups except during the reproduction cycle. Body weights were recorded weekly on F_0 rats, except during the reproduction phase, and food consumption was measured during wk 2 and 5, the week before each mating and the week after weaning of the F_{1a} litters. F_{1b} rats were weighed when started on the diet, during the week before mating and at sacrifice.

The F_0 rats were pair-mated when 3 and 7 months old to produce the F_{1a} and F_{1b} generations, respectively. Breeding-stock F_{1b} rats were selected at weaning from all available litters and pair-mated when 3 months old to produce the F_{2a} generation. F_{1b} rats on dietary levels of 0, 20 and 100 ppm Aroclor 1254 were mated a second time when 8 months old to produce the F_{2b} generation. Viability counts of offspring were made at birth, day 3, day 7 and day 21 (weaning). Litters were inspected daily for condition and the presence of dead pups. After the F_{1b} offspring had been weaned, ten adult F_0 rats of each sex were killed and their livers were weighed and fixed for histological examination. Following the weaning of the F_{2b} generation, for Aroclor 1254, and the F_{2a} generation, for Aroclor 1260, haematological values (total leucocyte count, haematocrit, haemoglobin and differential leucocyte count) were determined on ten adult F_{1b} rats of each sex at the dietary levels of 0, 20 and 100 ppm. These rats were then killed and weights of the spleen, heart, lungs, brain, kidneys, testes and liver were recorded. Tissues were fixed for microscopic study.

At lower dietary levels, haematology was not done and only the livers were weighed. At dietary levels of 5 ppm or less of both Aroclors, one male and one female weanling from each of ten litters from each generation were killed at 21 days of age and the livers were weighed. F_{2a} weanlings at dietary levels of 0, 20 and 100 ppm Aroclor 1254, and F_{1b} and F_{2a} weanlings at levels of 0, 20 and 100 ppm Aroclor 1260 were killed in the same manner and the livers were fixed for further study.

One-generation reproduction studies. Preliminary studies were conducted on both Aroclors at dietary levels of 0, 100 and 500 ppm. The experimental design was the same as that described for the two-generation studies, except that only ten females were used. This study was terminated after the F_{1b} offspring had been weaned. Rats fed 500 ppm Aroclor 1254 were bred only once. Haematology values and organ weights were determined and tissues were examined microscopically on the F_0 rats.

Post-implantation exposure studies. Stock rats 100 days old were pair-mated. Insemination was verified by microscopic inspection of a daily vaginal smear. Counting the day of insemination as day 0, the females were dosed by oral intubation on days 7–15 of gestation (a total of nine doses). To maintain a constant dose volume on a body-weight basis, Aroclor 1254 or 1260 was dissolved in peanut oil at appropriate concentrations and these formulations were administered at the rate of 5 ml/kg body weight, based on day 7 of pregnancy. Aroclor 1254 was given at dosage levels of 0 (peanut oil only), 10, 50 and 100 mg/kg/day. Aroclor 1260 was given at dosage levels of 0 and 100 mg/kg/day. The females were allowed to deliver and the litters were observed through weaning.

Tissue preparation. Tissues were fixed in buffered 4% formaldehyde solution and stained with haematoxylin and eosin for microscopic examination. An Oil Red O stain on fixed frozen sections was used on occasional livers to demonstrate lipids.

Statistics and calculations

LD_{50} values were calculated by the method of Litchfield & Wilcoxon (1949). A *t* test was used for comparing the litter sizes, organ weights, body weights and haematological parameters of the treated groups with those of the control groups. Survival percentages of offspring were compared by the Mann–Whitney *U* test as proposed by Weil (1970). Food consumption values were arrived at by averaging the consumption of both males and females over the five periods in which food was measured.

RESULTS

LD_{50} studies on Aroclor 1254 and 1260

The single-dose oral LD_{50} for Aroclor 1254 in weanling male rats was 1295 mg/kg with 95% confidence limits of 1136–1476 mg/kg. The lowest lethal dose tested was 1200 mg/kg, and 1000 mg/kg produced mild diarrhoea. Signs of toxicity at lethal levels were diarrhoea, depression and salivation, with death occurring in 1–3 days.

For Aroclor 1260, the single-dose oral LD_{50} in weanling males was 1315 mg/kg with 95% confidence limits of 1174–1473 mg/kg. The lowest lethal dose tested was 1000 mg/kg, 800 mg/kg produced no signs of toxicity. Rats given lethal doses developed diarrhoea and depression, with death occurring in 1–7 days.

Under the same test conditions the estimated oral LD_{50} for Aroclor 1254 and 1260 in adult rats was 4–10 g/kg (Kimbrough *et al.* 1972).

The single-dose iv LD_{50} for Aroclor 1254 in adult female rats was 358 mg/kg with 95%

confidence limits of 328–390 mg kg. The lowest lethal dose tested was 300 mg kg; 250 mg kg produced dyspnoea, depression, diarrhoea and salivation soon after dosing. Most rats died within 25–110 min, but at the highest dose (400 mg kg), death occurred as soon as 5 min after dosing in some animals. Rats that survived appeared normal after 48 hr. In the control group, which received the formulation vehicle only, one rat developed mild diarrhoea.

Preliminary one-generation reproduction studies on Aroclor 1254 and 1260

The reproduction summary for the Aroclor 1254 feeding studies is presented in Table 1. Exposure for 67 days (at the time of pair-mating) to 500 ppm Aroclor 1254 (37·0 mg kg day) resulted in fewer litters, smaller litter sizes and 100% mortality by day 3 in the F_{1a} pups. The survival of offspring of the rats fed 100 ppm (7·2 mg kg day) for 67 and 186 days before mating was reduced, only 85·9 and 68·1% of the pups surviving to weaning in the F_{1a} and F_{1b} generations, respectively. Litter sizes from both matings were smaller, but the difference was not statistically significant. The mean body weight of the 100 ppm pups at weaning was 7·8 and 10 g less than that of the controls for the F_{1a} and F_{1b} generations, respectively. This difference was probably dose-related, as the treated litters were smaller, but a statistical evaluation was not done. In general the pups appeared small, but otherwise were normal at weaning.

Aroclor 1260 fed at a dietary level of 500 ppm (35·4 mg kg day) for 67 and 186 days prior to mating markedly reduced live-litter size and survival-to-weaning in the F_{1a} and F_{1b} generations (Table 2). No effect on reproduction was observed in rats fed 100 ppm (6·0 mg kg day) in either the F_{1a} or F_{1b} generations.

The F_0 rats in the one-generation studies were sacrificed after exposure for 8 months. Organ-weight analysis indicated that the liver was the primary organ affected. As reported in the previous paper, the livers in male rats receiving either Aroclor 1254 or 1260 at dietary levels of 100 and 500 ppm were heavier than in controls; this was also observed in females fed Aroclor 1254 at levels of 100 and 500 ppm or Aroclor 1260 at 500 ppm (Kimbrough *et al.*, 1972). The testes-to-body weight ratios of F_0 rats fed Aroclor 1254 or 1260 at the 500 ppm level were greater than those of the controls ($P < 0{\cdot}025$). The difference in actual weight of the testes, however, was not significant. This observation may reflect the reduced body-weight gain observed in all F_0 rats fed the 500 ppm level of both Aroclors. No difference in other organ weights was found.

After 8 months exposure, terminal haematological values in F_0 rats indicated a reduction in haemoglobin and haematocrit ($P < 0{\cdot}005$) in both sexes fed 500 ppm Aroclor 1254 or 1260. The haematocrit was reduced in males ($P < 0{\cdot}05$) on 100 ppm Aroclor 1260, but haemoglobin values were normal in this group. The total leucocyte count was normal in rats fed Aroclor 1260, but there was a shift in the differential count. The number of lymphocytes was increased in females ($P < 0{\cdot}005$) fed 100 and 500 ppm Aroclor 1260 and in males ($P < 0{\cdot}05$) fed 500 ppm. A corresponding decrease ($P < 0{\cdot}05$) in polymorphonuclear leucocytes was observed. Differential values for rats fed Aroclor 1254 were normal, but an increase ($P < 0{\cdot}001$) in total leucocytes was observed in the 500 ppm females.

Two-generation reproduction studies on Aroclor 1254

F_0 rats fed 100 ppm Aroclor 1254 (7·6 mg kg day) for 62 or 188 days before mating produced smaller litters than the controls in both the F_{1a} and F_{1b} generations (Table 1). Survival-to-weaning was not affected in the F_{1a} offspring. Only 73·6% of the 100 ppm F_{1b} pups

Table 1. *Reproduction and pup survival in groups of rats fed Aroclor 1254*

Generation	Dietary level (ppm)	Parental exposure (days)†	No. of females mated	No. of litters		Litter size§		Total pups group				Survival (l''_a)	Mean body weight at weaning (g)
								Born (found)		Alive at			
				Born‡	Weaned	At birth	At weaning	Dead	Alive	Day 3	Weaning		
F_{1a}	0	67	10	8	7	11·1	10·6	3	89	86	85	95·5	39·2
	100	67	10	9	8	9·4	8·1	6	85	78	73	85·9	31·4
	500	67	10	4 (2)	0	4·0*		5	8	0	0	0	
	0	62	20	17	17	12·4	11·8	0	211	205	201	95·3	38·7
	20	62	20	19	19	11·7	11·5	3	222	220	219	98·6	38·3
	100	62	20	19	19	10·7*	10·3	10	204	199	196	96·1	32·9
	0	67	20	18	18	11·2	11·1	2	202	200	200	99·0	38·5
	1	67	20	16	15	9·2	9·1	3	147	145	145	98·6	42·3
	5	67	20	17	17	10·8	10·8	1	184	184	183	99·5	42·1
F_{1b}	0	186	10	7	7	11·6	11·6	1	81	81	81	100	37·9
	100	186	10	8	6	11·8	8·0	0	94	74	64	68·1**	27·9
	0	188	20	17	17	12·3	11·1	2	209	194	189	90·4	37·1
	20	188	20	18	18	10·4*	10·1	1	188	187	181	96·3	39·0
	100	188	20	20	19	9·5**	7·0	6	189	164	139	73·6	32·1
	0	201	20	18	18	12·3	11·0	6	222	211	198	89·2	40·7
	1	201	20	18	17	11·1	9·8	3	200	194	176	88·0	38·0
	5	201	20	20	19	11·1	10·2	2	222	218	203	91·4	38·9
F_{2a}	0	129	20	18	18	12·5	12·2	0	225	221	220	97·8	35·5
	20	129	20	17	17	10·6*	10·1	5	181	174	171	94·5	36·8
	100	129	20	7 (2)	4	7·2**	5·6	7	36	29	28	77·8**	35·2
	0	125	20	19	19	11·7	11·5	3	223	220	218	97·8	37·4
	1	125	20	15	15	11·7	11·5	0	175	174	173	98·9	37·2
	5	125	19	17	17	12·1	11·7	1	205	199	198	96·6	35·7
F_{2b}	0	274	20	17	16	12·7	11·3	4	216	201	191	88·4	31·9
	20	274	20	12	12	9·6**	8·5	6	115	103	102	88·7	36·1
	100	274	20	4 (2)	2	3·5**	3·5	7	7	7	7	100·0	38·3

† Conception to mating for the parents of the F_{2a} and F_{2b} generations.
‡ Numbers in parentheses indicate numbers of litters in which no live offspring were found.
§ Number of live offspring live litter born.
One-generation study.
Values marked with asterisks differ significantly from the control value: * $P < 0{\cdot}05$; ** $P < 0{\cdot}005$.

survived to weaning, but this was not statistically significant ($P = 0.058$). Because of the poor condition of a number of the 100 ppm F_{1b} pups, all were held for 30-days post-weaning observation. Fifteen of these pups died within 30 days of weaning. The mean body weights of the 100 ppm F_1 generation pups from either mating was about 6 g less than the controls at weaning. This is probably significant, since the treated litters contained fewer pups. A reduction in litter size was also observed in the F_{1b} generation at 20 ppm (1·5 mg/kg/day).

F_{1b} rats bred after exposure for 129 or 274 days (conception to mating) to 20 or 100 ppm had smaller litters than the controls (F_{2a} and F_{2b} generations). A marked decrease in mating performance was observed in the 100 ppm group, in which only seven and four litters were born in the F_{2a} and F_{2b} generations, respectively. In each case two litters contained no viable offspring when first observed. The data also suggest a decrease in mating performance at 20 ppm, at which level only 12 F_{2b} litters were produced. Survival-to-weaning was reduced in the 100 ppm F_{2a} offspring. All 100 ppm F_{2b} offspring survived to weaning, but this is of little significance since only seven pups were found alive. No effect on reproduction was observed in rats fed 5 ppm (0·32 mg/kg/day) or 1 ppm (0·06 mg/kg/day) through two generations.

Although reproduction was not affected at lower dietary levels of PCBs, an increase in liver weight in 21-day-old weanlings was found at all levels tested (Table 2). At 5 ppm or higher, the liver-to-body weight ratios of weanlings were increased in both sexes in both

Table 2. *Mean liver weights of 21-day-old rats from parents fed Aroclor 1254 or 1260*

Aroclor	Generation	Dietary level (ppm)	Liver weight			
			Males		Females	
			g	% of body weight	g	% of body weight
1254	F_{1a}	0	1·40	3·69	1·38	3·83
		1	1·81*	4·01*	1·63	3·91
		5	1·92*	4·34*	1·86*	4·43*
	F_{1b}	0	1·58	3·69	1·58	3·84
		1	1·54	3·94*	1·49	3·92
		5	1·77	4·63*	1·72	4·75*
	F_{2a}	0	1·37	3·81	1·28	3·74
		20	2·26*	5·78*	2·23*	6·08*
		100	2·63*	6·97*	2·67*	7·10*
	F_{2a}	0	1·63	3·78	1·42	3·63
		1	1·49	3·74	1·36	3·69
		5	1·56	4·23*	1·38	4·09*
1260	F_{1a}	0	1·54	3·66	1·57	3·92
		5	1·73	4·18*	1·70	4·39*
	F_{1b}	0	1·59	3·73	1·55	3·93
		20	2·06*	5·04*	2·14*	5·18*
		100	2·59*	6·33*	2·59*	6·48*
	F_{1b}	0	1·35	3·59	1·29	3·70
		5	1·58	4·01*	1·52*	4·07*
	F_{2a}	0	1·65	3·82	1·64	4·02
		20	2·06*	5·21*	1·94	5·06*
		100	2·50*	6·12*	2·49*	6·85*
	F_{2a}	0	1·41	3·85	1·36	3·87
		5	1·66*	4·21*	1·60*	4·25*

Values marked with an asterisk differ significantly from the control value: * $P < 0.05$.

the F_1 and F_2 generations, but at 1 ppm the liver enlargement was observed only in the F_{1a} and F_{1b} male weanlings. No liver-weight increase was found in either sex of the 1 ppm F_{2a} weanlings.

In 10-month-old F_{1b} rats, an increase in liver-to-body weight ratios was observed in both sexes at 100 ppm, but at 20 ppm it was seen only in the males (Table 3). At 5 ppm or less no increase in liver weight was found in 6-month-old F_{1b} rats or in F_0 rats exposed for 10 months. Both sexes of F_0 rats fed 20 ppm or more Aroclor 1254 for 8 months had enlarged livers (Kimbrough *et al.* 1972). The testes-to-body weight ratios were increased ($P < 0{\cdot}001$) in 100 ppm F_{1b} adult males. Body-weight gains of adults in all the test groups were comparable to those of the controls except in the F_{1b} females at 100 ppm, which gained less weight than the controls (Table 3). Reduced haemoglobin ($P < 0{\cdot}005$) and haematocrit ($P < 0{\cdot}025$) were observed in female F_{1b} adults at 100 ppm, while F_{1b} males at this level had reduced ($P < 0{\cdot}05$) haemoglobin only. Haematological values at lower levels were normal. No dose-related signs of toxicity were observed in adult F_0 or F_{1b} rats fed 100 ppm or less.

Two-generation reproduction study on Aroclor 1260

No effect on reproduction was observed in rats fed Aroclor 1260 at levels of 100, 20 or 5 ppm (7·4, 1·5 and 0·39 mg/kg/day) through two generations (Table 4). The liver-to-body weight ratios of 21-day-old pups were increased at all dietary levels (Table 2). This effect was observed at the lowest level tested (5 ppm) in all generations.

An increase in liver-to-body weight ratios was observed at all dietary levels in F_{1b} males between 5 and 7 months old, but only at 100 ppm in the F_{1b} females (Table 3). After 8 months exposure, an increase in liver weight was observed at 20 and 100 ppm in F_0 males but not in females (Kimbrough *et al.* 1972). No effect on liver weight was found in either sex of F_0 rats fed 5 ppm for 8 months. The testes-to-body weight ratios of the 100 ppm F_{1b} males were increased ($P < 0{\cdot}05$); at 20 ppm the increase was not significant. Body-weight gain in all test groups was comparable to the control groups. Haematological values were normal in the 20 ppm F_0 adults and in F_{1b} adults at 100 ppm or less. No signs of toxicity were observed in any of the test animals.

Post-implantation exposure studies on Aroclor 1254 and 1260

The data on reproduction and survival of offspring from females dosed during organogenesis is summarized in Table 5. Nine oral doses of Aroclor 1254 (100 mg/kg/day) given on days 7–15 of gestation resulted in a decrease in the survival of the pups. At this dose only 30·1% of the offspring of the treated group survived to weaning, compared with 98·2% in the control group, and the mean body weight of the test pups was 7·1 g less than that of the controls at weaning. No effect was observed in the groups receiving 50 mg/kg/day or less.

No effect on reproduction or survival was observed with Aroclor 1260 given at a rate of 100 mg/kg/day.

Summary of pathology after treatment with Aroclor 1254 and 1260

A detailed account of the liver pathology in the F_0 rats fed Aroclor 1254 or 1260 at 20 ppm or higher dietary levels has been published (Kimbrough *et al.* 1972). Of the rats considered in the current report, similar changes were observed in the liver of adult rats, particularly in the F_{1b} generations at the higher dietary levels. The incidence of the various

Table 3. *Body weight, liver weight and liver pathology of F_0 and F_{1b} adult rats fed Aroclor 1254 or 1260*

Generation	Sex	Exposure (days)	Dietary level (ppm)	Terminal body weight (g)	Liver weight		No. of livers examined	Liver pathology (no. of livers affected)						
					g	% of body weight		Enlarged hepatocytes	Inclusions	Foamy cytoplasm	Pigment	Fibrous strands	Adenofibrosis	Nodules
Aroclor 1254														
F_0	M	310	0	601	15·22	2·53	10	0	0	0	0	0	0	0
			1	602	15·88	2·60	10	0	0	0	0	0	0	0
			5	614	15·37	2·51	10	0	0	1	2	0	0	0
	F	313	0	370	10·34	2·80	10	0	0	0	1	0	0	0
			1	358	10·14	2·84	10	1	0	0	4	0	0	0
			5	362	10·66	2·94	10	4	1	0	3	0	0	0
F_{1b}	M	328	0	588	14·51	2·47	10	0	0	0	0	0	0	0
			20	602	16·11*	2·67*	8	8	3	7	1	0	0	0
			100	554	19·43*	3·54*	10	10	5	7	4	2	3	1
	F	328	0	349	9·92	2·85	10	0	0	0	0	0	0	0
			20	340	9·41	2·78	7	7	2	5	7	0	0	3
			100	300*	10·53	3·60*	10†	10	2	8	9	3	5	7
F_{1b}	M	190	0	475	11·13	2·34	10	0	0	0	0	0	0	0
			1	453	11·14	2·44	10	0	0	0	0	1	0	0
			5	495	12·19	2·46	10	2	0	2	0	1	0	0
	F	190	0	287	8·27	2·87	10	0	0	0	0	0	0	0
			1	294	8·44	2·86	10	1	1	0	0	4	0	0
			5	299	8·92	2·98	10	1	0	0	0	1	0	0
Aroclor 1260														
F_0	M	250	0	563	14·45	2·57	10	1	0	0	0	0	0	0
			5	567	15·06	2·65	10	3	0	2	0	0	0	0
	F	250	0	322	10·09	3·13	10	0	0	0	0	0	0	0
			5	331	10·80	3·25	10	1	0	0	0	0	0	0
F_{1b}	M	179	0	472	12·68	2·68	10	0	0	0	0	0	0	0
			20	494	14·72*	2·99*	10	8	5	4	0	0	0	0
			100	509	16·63*	3·27*	10	10	10	3	0	0	0	1
	F	179	0	297	9·66	3·27	10	0	0	0	0	0	0	0
			20	304	10·30	3·40	10	6	0	0	0	0	0	0
			100	304	11·14*	3·70*	10	9	0	1	5	2	1	2
F_{1b}	M	217	0	492	11·97	2·43	10	0	0	0	0	0	0	0
			5	505	13·16	2·60*	10	7	2	2	1	0	0	0
	F	217	0	279	7·93	2·84	10	0	0	0	0	0	0	0
			5	286	8·49	2·96	10	2	0	0	0	0	0	0

† Necrosis in two livers.
Values marked with an asterisk differ significantly from control group: * $P < 0.025$.

Table 4. *Reproduction and pup survival in groups of rats fed Aroclor 1260*

Generation	Dietary level (ppm)	Parental exposure (days)†	No. of females mated	No. of litters		Litter size‡		Total pups/group				Survival (%)	Mean body weight at weaning (g)
								Born (found)		Alive at			
				Born	Weaned	At birth	At weaning	Dead	Alive	Day 3	Weaning		
F_{1a}	0§	67	10	9	9	12·3	12·2	2	111	111	110	99·1	37·0
	100§	67	10	8	8	11·6	11·6	0	93	93	93	100·0	35·6
	500§	67	8	8	3	8·5**	3·3	10	68	55	26	38·2*	36·3
	0	68	20	19	19	11·7	11·2	2	223	217	213	95·5	40·9
	20	68	20	17	17	11·8	11·3	0	201	193	191	95·0	39·8
	100	68	20	17	17	11·6	11·2	1	197	192	191	97·0	38·0
	0	71	20	19	19	11·7	11·4	0	222	218	216	97·3	33·8
	5	71	20	18	18	12·1	11·8	1	217	213	212	97·7	38·5
F_{1b}	0§	186	10	9	8	12·1	10·3	1	109	96	93	85·3	40·9
	100§	186	9	5	5	11·0	10·8	0	55	54	54	98·2	36·2
	500§	186	8	6	2	6·7**	2·3	1	40	38	14	35·0	40·7
	0	187	20	17	17	11·1	11·0	6	189	189	187	98·9	40·8
	20	187	20	16	16	11·9	11·4	1	191	191	183	95·8	41·0
	100	187	20	13	13	10·4	10·1	5	135	131	131	97·0	39·2
	0	188	20	17	16	10·3	9·2	10	175	160	156	89·1	36·1
	5	188	19	16	15	11·2	10·6	2	179	171	170	95·0	39·3
F_{2a}	0	128	20	18	18	10·9	10·3	1	197	187	185	93·9	40·3
	20	128	20	19	19	11·3	11·1	1	215	213	211	98·1	38·7
	100	128	20	20	20	10·1	9·9	1	201	200	197	98·0	38·6
	0	127	20	13	13	11·8	11·6	1	153	152	151	98·7	34·8
	5	127	20	18	18	11·8	11·6	0	213	212	209	98·1	38·5

† Conception to mating for parents of the F_{2a} generation.
‡ Numbers of live offspring/live litter born.
§ One-generation study.
Values marked with asterisks differ significantly from the control value: $*P < 0.025$; $**P < 0.001$.

Table 5. *Reproduction and survival of pups from dams dosed orally with Aroclor 1254 or 1260 on days 7–15 of pregnancy*

Dose (mg/kg/day)	No. of females treated	No. of litters		Litter size†		Total pups/group				Survival (%)	Mean body weight at weaning (g)
						Born (found)		Alive at			
		Born	Weaned	At birth	At weaning	Dead	Alive	Day 3	Weaning		
Aroclor 1254											
0	9	9	9	12·3	12·1	0	111	110	109	98·2	37·9
100	9	7	5	11·9	3·6	8	83	64	25	30·1*	30·8
0	10	10	10	11·9	11·8	0	119	119	118	99·1	40·6
10	9	9	9	12·1	12·0	0	109	109	108	99·1	37·6
50	10	10	10	13·4	12·7	0	134	131	127	94·8	30·5
Aroclor 1260											
0	12	12	12	12·0	11·5	4	144	140	138	95·8	33·9
100	12	12	11	11·7	10·3	10	140	135	124	88·6	33·9

† No. of live offspring/litter born.
The value marked with an asterisk differs significantly from the control value: $^*P < 0{\cdot}001$.

changes is given in Table 3. Enlargement of the liver cells, involving primarily an increase of the cytoplasm surrounding the nucleus, was the most consistent finding and was occasionally observed at the 5 and 1 ppm dietary levels. Inclusions within the cytoplasm, which have also been referred to by some authors (Bennett, Drinker & Warren, 1938; Miller, 1944) as hyaline bodies, were observed more frequently in the males than in the females and were only occasionally noticed at the lower dietary levels. At 5 and 1 ppm the cytoplasm of many liver cells was vacuolated or foamy. According to the studies reported previously (Kimbrough *et al.* 1972), these changes were indicative of lipid accumulation. They were more pronounced at the higher dietary levels of 20 and 100 ppm. Adenofibrosis, which was also described in the previous paper, was observed only at the 100 ppm dietary level.

Two additional findings made in adult F_{1b} rats had not been observed previously in the F_0 rats. One was the occasional occurrence of strands of fibrous tissue which traversed the liver. In addition, hepatic nodules measuring up to 0·5 cm in diameter were observed in occasional livers of F_{1b} adults fed 100 ppm Aroclor 1254 or 1260. In addition, of the seven livers studied from F_{1b} adults fed 20 ppm Aroclor 1254, three showed one or more hepatic nodules. The hepatic nodules consisted of enlarged liver cells, which formed well-organized hepatic cords. The cytoplasm of many of these liver cells, which often stained somewhat lighter than the surrounding liver tissue, contained one or more inclusions. The nuclei of the liver cells within the hepatic nodules appeared normal (Fig. 1).

Apart from the findings in the livers, occasional rats of the control as well as the exposed groups showed mild-to-moderate chronic pyelonephritis. The incidence in the exposed group did not differ from that of the controls. One and two female rats fed 20 and 100 ppm Aroclor 1260, respectively, and two male rats fed 100 ppm Aroclor 1254 showed hyperplasia of the thyroid. Hyperplasia of the thyroid was not observed in the controls, but occasionally it does occur spontaneously in our rats. Whether this low incidence of hyperplasia of the thyroid is related to the consumption of Aroclor cannot be established with certainty from this study. No other morphological changes were observed in any of the other organs, including the testes.

The livers of F_{1b} weanlings from the group given the 20 ppm level of Aroclor 1254 showed slight enlargement of the liver cells but no other morphological changes. The livers of ten F_{1b} weanling rats at the 100 ppm level of Aroclor 1254 showed marked enlargement of the liver cells, which had either foamy or vacuolated cytoplasm. At 20 ppm, the livers of ten males and ten female F_{2a} weanling rats showed greatly enlarged liver cells, and the cytoplasm was vacuolated in most instances. Oil Red O stain on a few of these livers showed that vacuolization was due to lipid accumulation. Similar findings were made in four male and four female F_{2a} weanlings at the 100 ppm level of Aroclor 1254.

The livers of ten male and ten female F_{1b} weanling offspring from dams fed 20 ppm Aroclor 1260 had normal livers on microscopic examination, while those at 100 ppm showed enlarged and vacuolated hepatocytes. The livers of ten of 20 F_{2a} weanlings at the 20 ppm level of Aroclor 1260 and 17 of 19 livers of F_{2a} weanlings at the 100 ppm level showed enlarged cells and vacuolated cytoplasm.

At the lower dietary levels of both Aroclor 1254 and 1260 (5 ppm or less), livers of weanling rats were only occasionally examined in the different generations. The only microscopic change observed consisted of slightly enlarged hepatocytes in some of the livers.

DISCUSSION

The results of our studies confirm the capability of some commercial PCB mixtures to alter reproductive processes in mammals. Our data are in general agreement with those of Keplinger, Fancher & Calandra (1971), who observed effects on mating performance and/or pup survival in rats fed 100 ppm Aroclor 1242 or 1254, but found no effect at the same dietary level of Aroclor 1260. In our study, 20 ppm Aroclor 1254 affected litter size and probably decreased mating performance in the second mating of the F_{1b} rats. The trend suggested the possibility that even lower levels might affect reproduction in subsequent generations, but M. L. Keplinger (personal communication 1973) found no effect through three generations in rats fed 10 ppm Aroclor 1254. Reproductive failure in mink exposed to PCBs has also been reported (Ringer *et al.* 1972). Although actual PCB consumption on a mg/kg basis was not reported, the mink is apparently more sensitive to PCB exposure than the rat, since levels of 1 and 5 ppm Aroclor 1254 affected reproduction after only 4 months' exposure. In the present study in rats, fewer pups per litter were observed at dietary levels of 500 ppm Aroclor 1260 and of 20 ppm or more of Aroclor 1254. Reported litter sizes are based on the pups found, but since it is common for the dam to eat dead or defective offspring, the reduced litter size probably reflects decreased fertility and/or increased perinatal and foetal mortality.

Aroclor 1254 (100 mg/kg/day) given to pregnant rats during gestation produced no effect on the viability or morphology of foetuses examined on day 22 of gestation (Villeneuve, Grant, Khera, Clegg, Baer & Phillips, 1971). In the present study under a similar dosing regimen, dams dosed orally during gestation with Aroclor 1254 (100 mg/kg/day) gave birth to litters that were grossly normal, but only 30·1% of the pups survived to weaning. Since PCBs are excreted in milk (Curley, Burse & Grim, 1973; Fries, 1972), it is possible that PCB ingestion via the milk contributed to the increased mortality of pups observed in both the post-implantation and dietary-exposure studies. The milk of rats 11 days after the last of nine daily doses of Aroclor 1254 (50 mg/kg/day) contained 66 ppm of PCB-derived material (Curley *et al.* 1973). Kimbrough *et al.* (1972) estimated the oral LD_{50} for Aroclor 1254 and 1260 at 4–10 g/kg in adult rats. From the oral LD_{50} values in weanlings in the present study (about 1300 mg/kg), it is evident that the Aroclor mixtures tested are considerably more toxic to immature rats than to adults. Although we have no acute toxicity data for PCBs in neonates, nor figures on their milk consumption, it is possible that lacteal exposure could approach a toxic level in suckling rats at the higher exposure levels.

In both the dietary and post-implantation studies, direct PCB exposure occurred *in utero*, since PCBs have been shown to cross the placenta (Curley *et al.* 1973; Grant, Villeneuve, McCully & Phillips, 1971). PCBs have been reported in eggs (Peakall, 1971) and embryonic mortality has been observed in birds treated experimentally with commercial PCB mixtures (Dahlgren *et al.* 1972; Peakall, Lincer & Bloom, 1972). Dahlgren *et al.* (1972) also reported a decrease in survival and a depression of weight gain in 6-wk-old pheasant chicks from hens treated with Aroclor 1254. In the present studies, similar effects were observed in young rats at the higher exposure levels of Aroclor 1254. Besides a reduction in observed litter size, there was additional evidence of late foetal mortality in the group given 100 ppm Aroclor 1254, no live offspring being found in four of the F_2 litters. Thus it is apparent that some PCB mixtures affect the embryo in both mammalian and avian systems, although the mechanism of this effect remains undefined.

Although the suckling rats ate some of the Aroclor-treated diet for 4–5 days before weaning, it is probable that the observed increase in liver weight in 21-day-old rats was

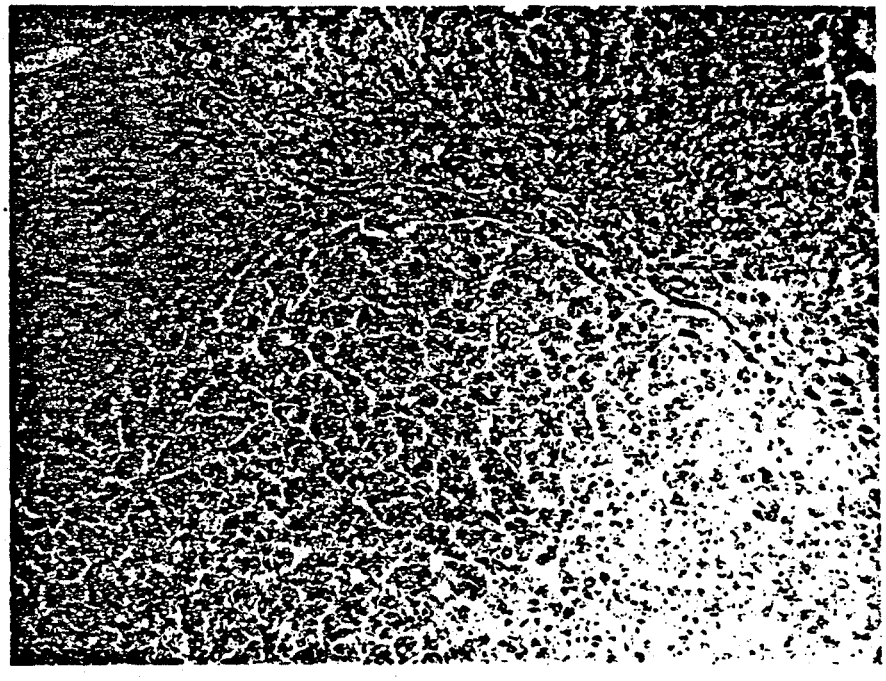

Fig. 1. Section of rat liver, showing a hepatic nodule measuring 0·2 cm in diameter surrounded by normal liver cells (arrow at margin of hepatic nodule). The hepatocytes within the nodule are larger than those of the surrounding tissue and stain less well. Haematoxylin and eosin × 100.

due to PCB exposure via the mothers' milk. Curley *et al.* (1973) also observed a liver-weight increase in weanlings from dams dosed only during gestation. When Aroclor 1254 was given during pregnancy to rabbits, maternal liver weight was increased but not foetal liver weight on day 29 of gestation (Villeneuve *et al.* 1971), thus eliminating the possibility that placental transfer of PCB was the cause of the liver-weight increase seen in weanlings. When adult rats of the F_{1b} generation were killed after dietary exposure for 5–6 months, liver weights at the lower dietary levels were comparable to those in the controls except in males given 5 ppm Aroclor 1260. Presumably once exposure via the milk ceased, the livers returned to normal in spite of a continued low dietary intake of PCB. At a dietary level of 1 ppm Aroclor 1254, only the male weanlings of the F_{1a} and F_{1b} generations had enlarged livers. The absence of this effect in the F_{2a} weanlings is inconsistent. One explanation could be the credibility of the 1 ppm formulation. Problems inherent in consistently reproducing a 1 ppm formulation, as well as contamination of the basal diet with PCBs, are possible sources of error. Over the last 18 months this laboratory has analysed the basal diet for PCB contamination at 6-wk intervals. Most values have been less than 0·1 ppm, but occasionally values of up to 0·5 ppm have been found. The latter level of contamination would introduce a 50% error in a 1 ppm formulation. It is likely that 1 ppm in the parental diet is very near a threshold effect level for the observed liver-weight increase in weanling rats.

At the lower dietary levels, adverse effects were not observed in weanling rats, which exhibited only an increase in liver weight. However, Aroclor 1254 fed to 1-month-old rats at the 100 ppm dietary level (approximately 7·9 mg/kg/day) increased cytochrome *P*-450 and liver weight after 2 days and microsomal protein after 3 days, while a single oral dose of 10 mg/kg increased cytochrome *P*-450 after 24 hr (Goldstein, Hickman & Jue, 1973). A 50–70% increase in nitroreductase activity was observed with Aroclor 1254 and 1260 fed to rats at a dietary level of 0·5 ppm for 4 wk (Litterst, Farber, Baker & Van Loon, 1972). In view of these reports it is very probable that offspring in the reproduction studies, even those involving the lowest levels of PCBs, were subject to inductive effects.

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L'effet des biphényles polychlorurés sur la reproduction du rat

Résumé—On a étudié la reproduction, la pathologie et les indices d'intoxication aiguë chez des rats auxquels on administrait l'un ou l'autre des biphényles polychlorurés Aroclor 1254 et Aroclor 1260. Les rats qui recevaient l'Aroclor 1254 à raison de 20 ppm ou plus du régime avaient des nichées moins nombreuses que les animaux témoins aux générations F_{1b} et F_2. La dose de 100 ppm d'Aroclor 1254 a fait augmenter la mortalité dans la génération F_{1b} et fait nettement diminuer les performances d'accouplement des adultes de cette génération. Administré à raison de 500 ppm du régime, l'Aroclor 1260 a fait diminuer l'importance des nichées et le taux de survie des nichées F_1.

Des taux de 5 ppm d'Aroclor 1254 et de 100 ppm d'Aroclor 1260 sont restés sans effet sur la reproduction des rats soumis à ces régimes pendant deux générations. Le poids du foie a augmenté chez les mâles F_1 âgés de 21 jours au régime à 1 ppm d'Aroclor 1254 et chez les mâles et femelles F_1 et F_2 sevrés des groupes à 5 ppm ou plus d'Aroclor 1254 ou 1260. Des modifications histologiques du foie et des augmentations du poids de cet organe ont été observées chez les rats adultes qui recevaient les plus fortes doses. Des femelles gravides qui avaient reçu 100 mg d'Aroclor 1254/kg de poids vif et par jour du 7ème au 15ème jour de gestation ont mis bas des nichées à peu près normales, mais seulement 30,1% de ces jeunes ont survécu au sevrage. La reproduction et le taux de survie des jeunes n'ont pas été influencés par les doses de 50 mg d'Aroclor 1254 kg jour et de 100 mg d'Aroclor 1260/kg jour. Les valeurs DL_{50} orales chez les rats mâles âgés de 3–4 semaines étaient de 1295 mg/kg d'Aroclor 1254 et de 1315 mg kg d'Aroclor 1260. La LD_{50} intraveineuse d'Aroclor 1254 était de 358 mg/kg chez les femelles adultes.

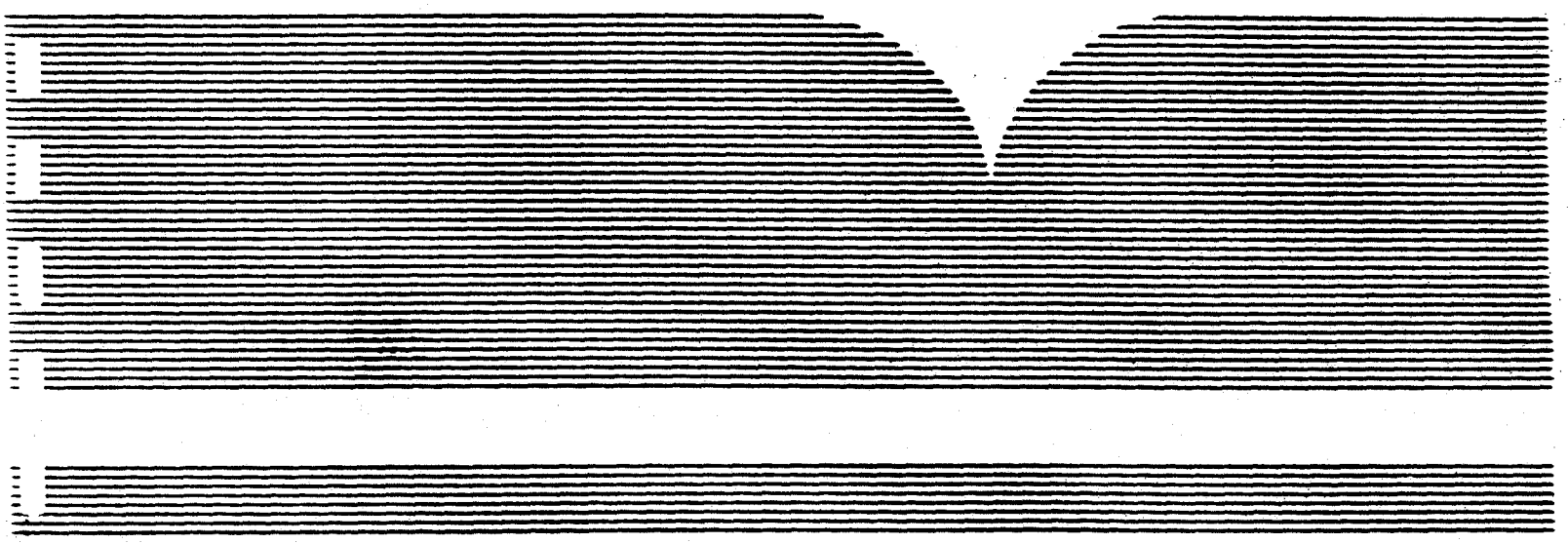
Der Einfluss von polychlorierten Biphenylen auf die Fortpflanzung der Ratte

Zusammenfassung – Vermehrung, Pathologie und akute Toxizität wurden an Sherman-Ratten untersucht, die den polychlorierten Biphenylen Aroclor 1254 und Aroclor 1260 ausgesetzt wurden. Ratten, die Aroclor 1254 in Konzentrationen von 20 ppm oder darüber im Futter erhielten, hatten weniger Junge je Wurf als die Kontrolltiere in den F_{1b}- und F_2-Generationen. Die Konzentration 100 ppm von Aroclor 1254 erhöhte die Sterblichkeit der F_{1b}-Nachkommen und setzte deutlich die Paarungsleistung der F_{1b}-Erwachsenen herab. Die Konzentration von 500 ppm Aroclor 1260 im Futter verminderte die Grösse der Würfe und verminderte das Überleben in den F_1-Würfen. Die Konzentrationen von 5 ppm Aroclor 1254 und 100 ppm Aroclor 1260 hatten keinen Einfluss auf die Fortpflanzung von Ratten, die ihnen über zwei Generationen ausgesetzt waren. Das Lebergewicht war bei 21 Tage alten männlichen abgesetzten F_1-Tieren bei der Konzentration von 1 ppm Aroclor 1254 und bei beiden Geschlechtern von abgesetzten F_1- und F_2-Jungen bei 5 ppm oder höheren Konzentrationen von Aroclor 1254 und 1260 erhöht. Histologische Veränderungen der Leber und erhöhtes Lebergewicht wurden bei erwachsenen Ratten beobachtet, welche die höheren Konzentrationen erhielten. Trächtige Ratten, die Aroclor 1254 in der Konzentration 100 mg/kg Tag vom 7. bis 15. Tag der Trächtigkeit erhielten, brachten äusserlich normale Würfe hervor, aber nur 30.1% der Jungen überlebten bis zum Absetzen. Die Fortpflanzung und das Überleben der Nachkommen wurden bei Dosierungen von 50 mg Aroclor 1254/kg/Tag oder 100 mg Aroclor 1260/kg/Tag nicht beeinflusst. Die oralen LD_{50}-Werte bei 3–4 Wochen alten männlichen Ratten betrugen 1295 und 1315 mg/kg bei Aroclor 1254 bzw. 1260. Die iv LD_{50} für Aroclor 1254 bei erwachsenen weiblichen Tieren betrug 358 mg/kg.

Bioassay of Aroclor (Trademark) 1254 for Possible Carcinogenicity
CAS No. 27323-18-8

National Cancer Inst, Bethesda, Md Carcinogenesis Program

October 1977



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BIOASSAY OF AROCLOR® 1254 FOR POSSIBLE CARCINOGENICITY

CAS No. 27323-18-8

NCI-CG-TR-38

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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16. Abstract

A bioassay of Aroclor 1254 for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats. Groups of 24 rats of each sex were administered Aroclor 1254 at one of three doses, either 25, 50, or 100 ppm, for 104-105 weeks. Matched controls consisted of groups of 24 untreated rats of each sex.

Survival among males, but not among females, showed a significant dose-related trend. Adequate numbers of animals of both sexes survived for meaningful statistical analyses of the incidences of tumors. The combined incidences of lymphomas and leukemias showed a significant dose-related trend in males. However, the direct comparisons of each treated group with those of the matched controls were not significant, and the tumors cannot clearly be related to treatment with Aroclor 1254. Hepatocellular adenomas and carcinomas were found in the treated groups, but not in the controls. Although the incidences of tumors were not significant, the occurrence of the hyperplastic nodules appeared to be related to treatment. (continued on attached sheet)

17. Key Words and Document Analysis. 17a. Descriptors

Aroclor 1254
Bioassay
Rodents
Rats

17b. Identifiers/Open-Ended Terms

17c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report) UNCLASSIFIED	21
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Attachment, Bioassay of Aroclor 1254 for Possible Carcinogenicity

16. ...
It is concluded that under the conditions of this bioassay, Aroclor 1254 was not carcinogenic in Fischer 344 rats; however, a high incidence of a spectrum of proliferative lesions of the liver in both male and female rats was related to treatment. In addition, the carcinomas of the gastrointestinal tract may be associated with treatment in both males and females.

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BIOASSAY OF

AROCLOR® 1254

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 78-838

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BIOASSAY OF AROCLOR® 1254 FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

CONTRIBUTORS: This report presents the results of the bioassay of Aroclor® 1254 for possible carcinogenicity, conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Stanford Research Institute, Menlo Park, California, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. R. R. Bates[1,2], D. C. L. Jones[3], D. P. Sasmore[3], C. W. Newell[3], and R. M. Elashoff[4], and Mr. W. E. Davis[3]. The principal investigator was Dr. D. C. L. Jones; the technical supervisor of animal treatment, observation, and data handling was Mr. W. E. Davis; necropsy and tissue fixation were supervised by Dr. D. P. Sasmore.

Histopathologic examinations were performed by Dr. H. Elster[5] and the diagnoses included in this report represent his interpretation. Neoplasms and compound-related hyperplastic lesions were reviewed by Drs. W. M. Busey[6] and J. F. Hardisty[6], who also prepared the interpretive pathology summary included in this report.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[7]. The statistical analyses were performed by Dr. J. R. Joiner[8], using methods selected for the bioassay program by Dr. J. J. Gart[9]. Chemicals used in this

bioassay were analyzed at Stanford Research Institute, and the analytical results were reviewed by Dr. C. W. Jameson[8].

This report was prepared at Tracor Jitco[8] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Drs. J. F. Robens and R. W. Fogleman, toxicologists; Dr. R. L. Schueler, pathologist; Ms. L. A. Waitz and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[9]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings:

Dr. Kenneth C. Chu
Dr. Cipriano Cueto, Jr.
Dr. J. Fielding Douglas
Dr. Dawn G. Goodman
Dr. Richard A. Griesemer
Mr. Harry A. Milman
Dr. Thomas W. Orme
Dr. Robert A. Squire[10]
Dr. Jerrold M. Ward

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[1]Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[2]Now with the Office of the Commissioner, Food and Drug Administration, Rockville, Maryland.

[3]Stanford Research Institute, Menlo Park, California.

[4]Department of Biomathematics, Center for the Health Sciences, University of California, Los Angeles, California.

[5]Department of Pathology, David M. Brotman Memorial Hospital, 3828 Hughes Avenue, Culver City, California.

[6]Experimental Pathology Laboratories, Inc., P.O. Box 474, Herndon, Virginia.

[7]EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

[8]Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

[9]Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[10]Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of Aroclor® 1254 for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats.

Groups of 24 rats of each sex were administered Aroclor® 1254 at one of three doses, either 25, 50, or 100 ppm, for 104-105 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104-105 weeks.

Mean body weights of males and females receiving mid and high doses and females receiving low doses of the chemical were consistently below those of the corresponding controls, beginning at about week 10 of the study. The decrease in survival among males, but not among females, showed a significant dose-related trend. Adequate numbers of animals of both sexes survived for meaningful statistical analyses of the incidences of tumors.

The combined incidences of lymphomas and leukemias showed a significant dose-related trend in males (controls 3/24, low-dose 2/24, mid-dose 5/24, high-dose 9/24, $P = 0.009$). However, the direct comparisons of each dosed group with those of the matched controls were not statistically significant, and the tumors cannot clearly be related to administration of with Aroclor® 1254.

Hepatocellular adenomas and carcinomas were found in the dosed groups, but not in the controls (males: mid-dose 1/24, high-dose 3/24; females: mid-dose 1/24, high-dose 2/24). Additionally, a high incidence of nonneoplastic hyperplastic nodules was noted in the dosed animals (males: controls 0/24, low-dose 5/24, mid-dose 8/24, high-dose 12/24; females: controls 0/23, low-dose 6/24, mid-dose 9/22, high-dose 17/24). Although the incidences of tumors were not significant, the occurrence of the hyperplastic nodules appeared to be related to administration of the chemical.

In the stomach, jejunum, or cecum, adenocarcinomas were observed in two dosed males and in two dosed females as well as a carcinoma in one dosed male. None of these lesions was found in control animals in this study. Historical incidences of these tumors at this laboratory (6/600 males [1%], 2/600 females [0.3%] suggest that the lesions -- although not statistically significant -- may be related to the administration of Aroclor® 1254.

It is concluded that under the conditions of this bioassay, Aroclor® 1254 was not carcinogenic in Fischer 344 rats; however, a high incidence of hepatocellular proliferative lesions in both male and female rats was related to administration of the chemical. In addition, the carcinomas of the gastrointestinal tract may be associated with administration of Aroclor® 1254 in both males and females.

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TABLES

FIGURES

I. INTRODUCTION

Aroclor® (CAS 27323-18-8; NCI C02664) is the registered trademark of the Monsanto Chemical Company for their polychlorinated biphenyls (PCBs). PCBs were developed in 1929 primarily for use as heat transfer fluids and dielectrics (insulators). Aroclor® 1254, a biphenyl containing approximately 54% chlorine, is a nonflammable heat transfer agent which functions in the range of 250-360°C (Hubbard, 1964; Poffenberger and Hubbard, 1965).

PCBs have been used in transformers and capacitors; as industrial fluids in hydraulic, gas turbine, and vacuum pumps; as lubricants and plasticizers (for flame retardation); and as additives in surface coatings, inks, papers, adhesives, sealants, pesticides, and dyes for carbonless duplicating paper (Hubbard, 1964; Broadhurst, 1972). These compounds tend to accummulate in the biosphere (Finklea et al., 1972). Because of direct and indirect human and animal exposure, food contamination, and environmental pollution from many of these uses, the marketing of PCBs has been markedly curtailed in recent years (EPA, 1977).

This bioassay of Aroclor® 1254 was conducted as a part of a larger study designed to assess the combined effects of a group of known or suspected carcinogens. Only the results of the study of the administration of Aroclor® 1254 are reported herein.

II. MATERIALS AND METHODS

A. Chemical

Aroclor® 1254 was obtained in a single batch (Lot No. KB01-604) from the Monsanto Chemical Company, St. Louis, Missouri. The identity and relative purity of the test chemical were confirmed at Stanford Research Institute. Elemental analyses (C, H, Cl) indicated 54.67% chlorine. Gas-liquid chromatography and mass spectroscopy showed that the Aroclor® 1254 contained at least 18 isomers of polychlorinated biphenyls ranging from 4 to 7 chlorine atoms per molecule. Identity was confirmed by nuclear magnetic resonance, infrared, and ultraviolet spectra, which were in agreement with the structure. No attempt was made to identify or quantitate impurities.

The chemical was stored at room temperature in 1-gallon amber glass jars.

B. Dietary Preparation

All diets were formulated every 2 weeks using Low Fat Lab Chow® (Ralston Purina Co., St. Louis, Mo.). A stock diet was first prepared by hand mixing a weighed amount of the Aroclor® 1254 with corn oil (Staley Manufacturing Co., Orange, Calif.) and adding this mixture to a small amount of feed which was also mixed by

hand. More corn oil and feed were then added to give a final concentration of 3,000 ppm Aroclor® 1254 and 3% corn oil and then machine mixed in a Hobart blender for 30 minutes. Each stock diet was analyzed for content of Aroclor® 1254 by a method involving extraction, Florisil® chromatography, and quantitation by gas-liquid chromatography. Concentrations of 3,000 ppm ± 10% were considered acceptable for use in preparing the test diets. Aroclor® 1254 at 3,000 ppm in the stock diet was found to be stable when held in rat feeders at room temperature for a 2-week period.

To obtain test diets having appropriate concentrations of Aroclor® 1254, the stock diet was diluted, as required, with control diet containing 3% corn oil and mixed in a Hobart blender. The stock and test diets were stored at room temperature in covered plastic containers.

C. Animals

Male and female Fischer 344 rats, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were obtained from Simonsen Laboratory, Gilroy, California. On arrival at the laboratory, all animals were quarantined for 2 weeks as an acclimation period. Following this period, all males gaining less than 25

grams, all females gaining less than 15 grams, and all unhealthy animals were culled. The remaining animals were assigned to cages, one per cage, until each cage contained three animals. Cages were then numbered and assigned to control and treated groups using a computer-generated randomization table. Rats were ear-clipped for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature was maintained at 22°C with a range of 21-24°C, and the relative humidity was maintained at approximately 45%. The room air was changed 10 times per hour and was maintained under positive pressure to the access halls. Fluorescent lighting provided illumination 12 hours per day. Food and water were available ad libitum. Drinking water was softened, filtered, sterilized with ultraviolet light, and supplied by means of an automatic watering system.

The rats were housed three per cage in polycarbonate cages equipped with disposable polyester woven filter tops. Autoclaved hardwood chips (Iso-Dri®, Becton, Dickinson, and Carworth, Warrensburg, N.Y.) were used as bedding. The cages were changed, washed, and provided with fresh bedding twice per week. Filter tops were replaced once per month.

Rats fed Aroclor® 1254 were housed in the same room as rats treated with aflatoxin B_1 (CAS 1162-65-8), lead (II) acetate (CAS 301-04-2), hexachlorophene (CAS 70-30-4), or dieldrin (CAS 60-57-1) in the feed.

E. Subchronic Studies

Subchronic feeding studies were conducted with male and female Fischer 344 rats to estimate the maximum tolerated dose of Aroclor® 1254, on the basis of which low, mid, and high concentrations (hereinafter referred to as "low doses", "mid doses", and "high doses") were determined for administration in the chronic studies. In the subchronic studies, Aroclor® 1254 was added to feed in concentrations of 25, 50, 100, 200, or 400 ppm. Treated and control groups each consisted of 15 male and 15 female rats. The chemical was provided in feed to the treated groups for 8 weeks.

The animals receiving 400 ppm were inactive, had occasional diarrhea and tremors, and failed to gain weight. At this dose 4/15 males and 1/15 females died. Enlarged livers were observed on gross examination, and histologically atypical hyperplasia was observed. At 200 ppm, body weights for both males and females were approximately 70% of those of the controls, and mild hepatocellular pleomorphism was seen histologically in the livers.

Rats treated with 25 ppm Aroclor® 1254 had enlarged livers, but no evidence of histologic abnormalities. Weight gain in all animals treated at doses lower than 200 ppm was comparable to that in controls, and there was no mortality below 400 ppm. The low, mid, and high doses for the chronic studies were set at 25, 50, and 100 ppm.

F. Design of Chronic Studies

The design of the chronic studies is shown in table 1.

G. Clinical and Pathologic Examinations

All animals were observed daily for signs of toxicity and palpated for masses at each weighing. Animals were weighed individually every other week for 12 weeks, and once every fourth week for the remainder of the study. Animals that were moribund at the time of clinical examination were killed and necropsied.

The pathologic evaluation consisted of gross examination of major organs and tissues from killed animals and from animals found dead. The following tissues were routinely examined microscopically from both treated and control animals: lungs and bronchi, spleen, liver, testes, pituitary, kidney, and brain. In addition, sections of stomach, urinary bladder, thyroid, uterus, and ovary were examined in a majority of the controls; these

Table 1. Design of Aroclor® 1254 Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Aroclor® 1254 in Diet[b] (ppm)	Time on Study	
			Treated[c] (weeks)	Untreated (weeks)
Males				
Matched-Control	24	0		105
Low-Dose	24	25	105	
Mid-Dose	24	50	105	
High-Dose	24	100	105	
Females				
Matched-Control	24	0		105
Low-Dose	24	25	104-105	
Mid-Dose	24	50	104-105	
High-Dose	24	100	105	

[a]All animals were 53 ± 2 days of age when placed on study.

[b]All diets contained 3% corn oil.

[c]All animals were started on study within 2 days of each other.

tissues were taken from treated rats only if a lesion was found at necropsy. Occasionally, additional tissues were examined microscopically. Gross lesions from all animals were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few of the tissues selected by design from some animals were not examined, particularly from those animals that died early. Thus, the number of animals from which particular organs or tissues were microscopically examined varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals necropsied (denominator).

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a

significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the

first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each treated group compared to its control was calculated

from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit

indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS

A. Body Weights and Clinical Signs

Beginning at about week 10 for the high-dose groups and about week 20 for the mid-dose groups, mean body weights of both male and female rats fed Aroclor® 1254 at the doses used in this bioassay were lower than those of the controls (figure 1). Mean body weights of low-dose males appeared comparable to those of controls throughout the study, while mean body weights of low-dose females were lower during the second year of the study. At week 30, an intercurrent respiratory infection in the colony caused weight loss, but no deaths; animals recovered within 30 days without treatment for the infection.

Clinical signs associated with administration of Aroclor® 1254 included alopecia, amber-colored urine, facial edema, exophthalmos, and cyanosis. These signs were apparent among the high-dose groups beginning at week 72 and among the mid-dose groups at week 104 of the study.

B. Survival

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed Aroclor® 1254 in the diet

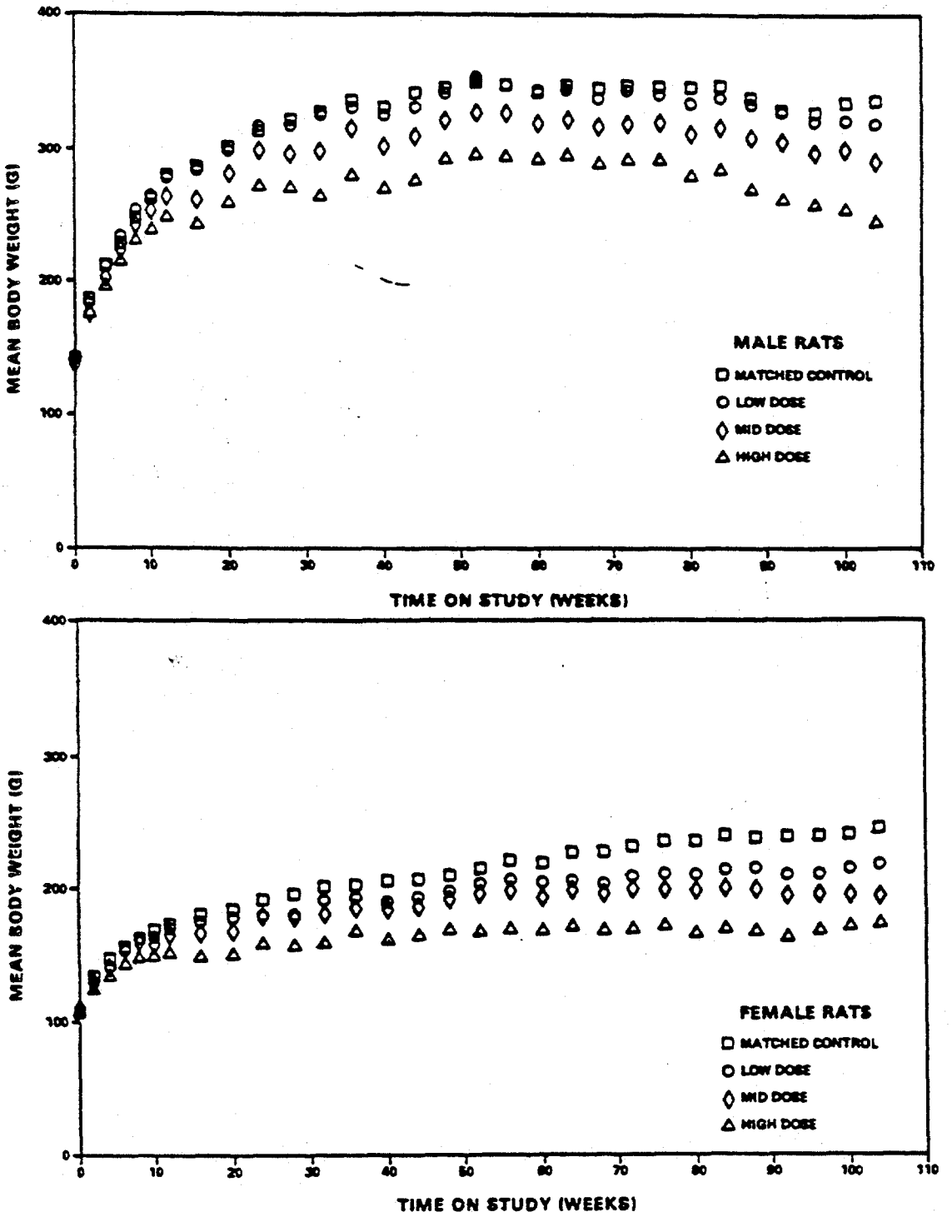

Figure1. Growth Curves for Rats Fed Aroclor® 1254 in the Diet

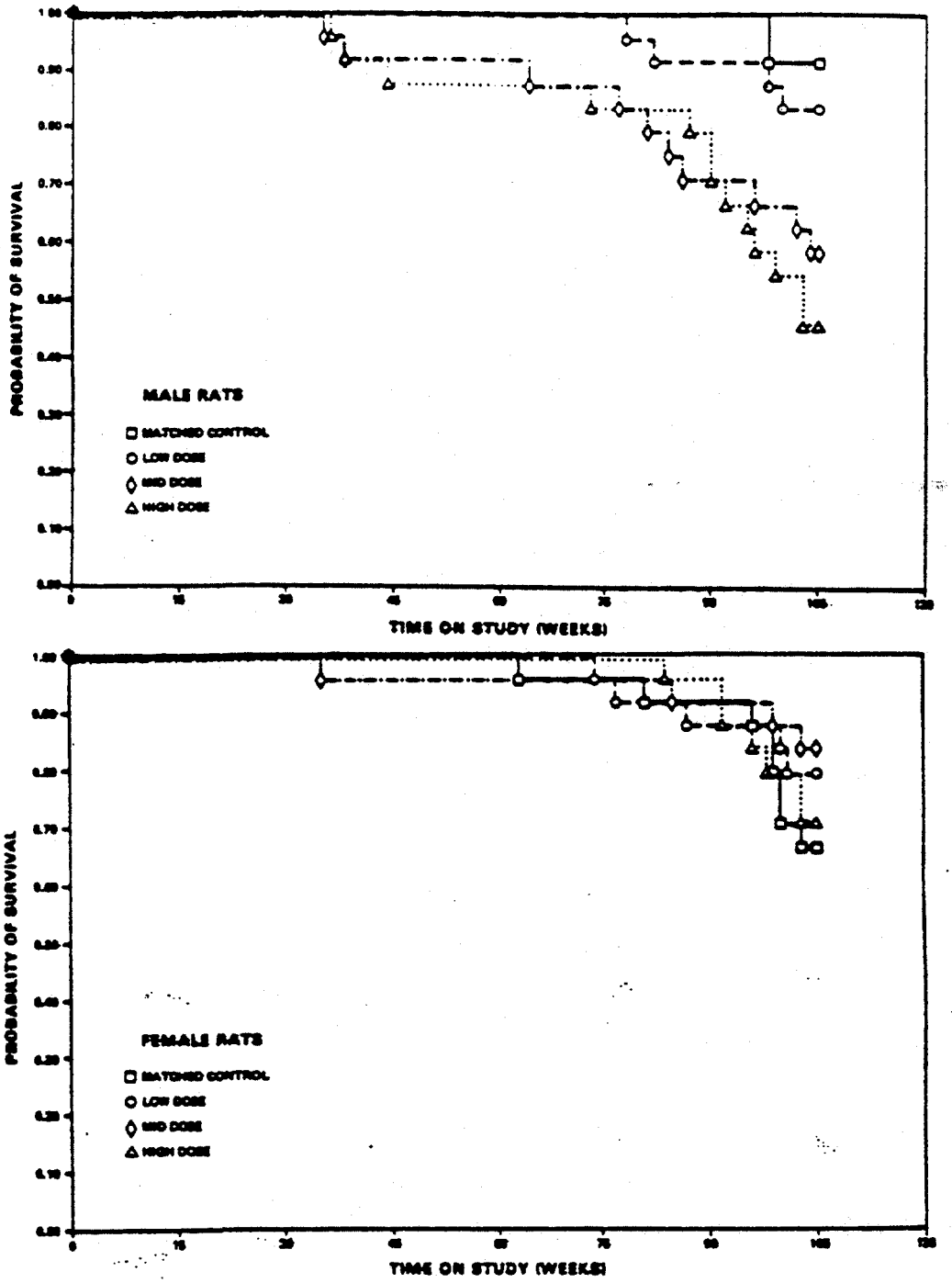

Figure 2. Survival Curves for Rats Fed Aroclor® 1254 in the Diet

at the doses used in this study, together with those of the controls, are shown in figure 2.

For males, the result of the Tarone test for positive dose-related trend in mortality over the period is significant ($P < 0.001$); 92% of the control, 83% of the low-dose, 58% of the mid-dose, and 46% of the high-dose rats survived to the end of the study. Among females, the Tarone test showed a probability level greater than 0.05. In females, 67% of the control, 79% of the low-dose, 83% of the mid-dose, and 71% of the high-dose rats survived to termination of the study. Sufficient numbers of rats of both sexes were available for meaningful statistical analyses of the incidences of late-developing tumors.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix B, tables B1 and B2.

A variety of neoplastic processes were observed in both the control and treated rats, and, with the exception of the liver, the incidences of these neoplasms were comparable in the control and treated groups. Interstitial-cell tumors of the testes were present in the majority of control and treated males. The next most frequently observed neoplasm was leukemia of either the

granulocytic or lymphocytic type, and it involved multiple organs. The incidence of this neoplastic process was comparable in the control and treated groups. The following neoplasms were also present in some control and treated rats but without compound association: squamous-cell carcinomas of the skin, alveolar/bronchiolar adenomas of the lung, and uterine endometrial stromal polyps.

No proliferative lesions of the hepatocytes were found in the control animals in the study. The incidence of these proliferative lesions among treated animals was as follows:

	MALES			FEMALES		
	Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
Number of Animals Necropsied	(24)	(24)	(24)	(24)	(22)	(24)
Nodular Hyperplasia	5	8	12	6	9	17
Adenoma, NOS*	0	0	1	0	1	2
Hepatocellular Carcinoma	0	1	2	0	0	0
Total Incidence	5	9	15	6	10	19

*Not otherwise specified

The areas of nodular hyperplasia appeared to be microscopically similar to what is currently termed "focal areas of cellular

alteration" (Squire and Levitt, 1975). Neither this lesion nor any hepatocellular adenomas or carcinomas were diagnosed in the controls. The hepatocellular carcinomas were characterized microscopically by large foci of proliferating hepatocytes involving several lobules. These hepatocytes were bizarre in appearance, sometimes containing two or more nuclei. The sinusoidal architecture was lost, and frequently mitotic figures were present. These neoplasms compressed the surrounding normal liver tissue, and the cell plates usually were three to five cells in thickness. The hepatocellular adenomas were characterized by large foci involving several lobules of swollen, severely vacuolated hepatocytes still maintaining the general sinusoidal architecture of the liver. In general, the foci of nodular hyperplasia involved two or more hepatic lobules and contained hepatocytes whose tinctorial properties were distinctly different from those of the surrounding liver tissue. Occasionally, these foci would contain severely vacuolated hepatocytes, and in some instances, there were small foci of basophilic hepatocytes.

The results of the histopathologic examination indicate that the administration of Aroclor® 1254 at the three doses used in this study had an effect with respect to proliferative lesions of the liver and gastrointestinal tract. There were three hepatocellular carcinomas in male rats and a dose-related increase in