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# Provisional Peer Reviewed Toxicity Values for

# Bifenox
(CASRN 42576-02-3)

Superfund Health Risk Technical Support Center
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## Acronyms and Abbreviations

| | |
|---|---|
| bw | body weight |
| cc | cubic centimeters |
| CD | Caesarean Delivered |
| CERCLA | Comprehensive Environmental Response, Compensation and Liability Act of 1980 |
| CNS | central nervous system |
| cu.m | cubic meter |
| DWEL | Drinking Water Equivalent Level |
| FEL | frank-effect level |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| g | grams |
| GI | gastrointestinal |
| HEC | human equivalent concentration |
| Hgb | hemoglobin |
| i.m. | intramuscular |
| i.p. | intraperitoneal |
| IRIS | Integrated Risk Information System |
| IUR | inhalation unit risk |
| i.v. | intravenous |
| kg | kilogram |
| L | liter |
| LEL | lowest-effect level |
| LOAEL | lowest-observed-adverse-effect level |
| LOAEL(ADJ) | LOAEL adjusted to continuous exposure duration |
| LOAEL(HEC) | LOAEL adjusted for dosimetric differences across species to a human |
| m | meter |
| MCL | maximum contaminant level |
| MCLG | maximum contaminant level goal |
| MF | modifying factor |
| mg | milligram |
| mg/kg | milligrams per kilogram |
| mg/L | milligrams per liter |
| MRL | minimal risk level |
| MTD | maximum tolerated dose |
| MTL | median threshold limit |
| NAAQS | National Ambient Air Quality Standards |
| NOAEL | no-observed-adverse-effect level |
| NOAEL(ADJ) | NOAEL adjusted to continuous exposure duration |
| NOAEL(HEC) | NOAEL adjusted for dosimetric differences across species to a human |
| NOEL | no-observed-effect level |
| OSF | oral slope factor |
| p-IUR | provisional inhalation unit risk |
| p-OSF | provisional oral slope factor |
| p-RfC | provisional inhalation reference concentration |
| p-RfD | provisional oral reference dose |
| PBPK | physiologically based pharmacokinetic |

| | |
|---|---|
| ppb | parts per billion |
| ppm | parts per million |
| PPRTV | Provisional Peer Reviewed Toxicity Value |
| RBC | red blood cell(s) |
| RCRA | Resource Conservation and Recovery Act |
| RDDR | Regional deposited dose ratio (for the indicated lung region) |
| REL | relative exposure level |
| RfC | inhalation reference concentration |
| RfD | oral reference dose |
| RGDR | Regional gas dose ratio (for the indicated lung region) |
| s.c. | subcutaneous |
| SCE | sister chromatid exchange |
| SDWA | Safe Drinking Water Act |
| sq.cm. | square centimeters |
| TSCA | Toxic Substances Control Act |
| UF | uncertainty factor |
| µg | microgram |
| µmol | micromoles |
| VOC | volatile organic compound |

# PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR BIFENOX (CASRN 42576-02-3)

## Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

## Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

## Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

# INTRODUCTION

Bifenox is not listed on IRIS (U.S. EPA, 2006), the HEAST (U.S. EPA, 1997a), or the Drinking Water Standards and Health Advisories list (2004). The U.S. EPA Office of Pesticide Programs (U.S. EPA, 1997b) derived a chronic RfD of 0.15 mg/kg-day for bifenox based on **a** NOAEL of 15 mg/kg-day in a chronic study in dogs fed a dietary concentration of 600 ppm for two years, and an uncertainty factor of 100 (10 for interspecies extrapolation and 10 for susceptible populations). No occupational exposure limits have been established for bifenox by ACGIH (2005), NIOSH (2006) or OSHA (2006). As a diphenyl ether herbicide, bifenox was registered under FIFRA in 1975 and subsequently cancelled (U.S. EPA, 1994a, 1998). A Pesticide Background Statement by U.S.D.A. (1987), a Summary of Toxicology Data by the California Environmental Protection Agency's Department of Pesticide Regulation (CDPR, 1988), and the Office of Pesticide Programs' OPPIN database were consulted for data on bifenox. No relevant documents were included in the CARA list (U.S. EPA, 1991, 1994b) or in the Health Canada First or Second Priority List Assessments (Health Canada 2006a, 2006b). ATSDR (2006), IARC (2006) and WHO (2006) have not reviewed the toxicity of bifenox. Bifenox is not listed in the NTP (2006) management status report. In the fall of 2002, literature searches were conducted for the period from 1965 to September 2002 to identify data relevant to a carcinogenicity assessment for bifenox. The following databases were searched: TOXLINE

(including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS. Update literature searches were conducted in November 2005 for the period of September 2002 to November 2005 in TOXLINE special (including NTIS updates) and TOXCENTER (BIOSIS subfile). Databases searched in November 2005 without date limitations included CCRIS, HSDB, GENETOX, and RTECS. Additionally, updated searches of MEDLINE (plus PubMed cancer subset, which replaces CANCERLIT) and Current Contents were performed for May 2005 to April 2006.

## REVIEW OF PERTINENT DATA

### Human Studies

No data were located regarding the oral or inhalation toxicity or carcinogenicity of bifenox in humans.

### Animal Studies

*Oral Exposure*

A four-week dietary study in rats was conducted by Huntingdon Research (1984) in order to determine appropriate dietary bifenox levels to use in long-term studies. Six groups of Sprague-Dawley rats (5/sex/group) were administered bifenox technical (purity and feed analysis not stated) in the diet at concentrations of 0, 1000, 2000, 3500, 5000 or 10,000 ppm. The study authors used observed values for body weight and food consumption to estimate mean doses of 0, 96, 190, 353, 495 or 904 mg/kg-day for male rats and 0, 105, 213, 372, 536 or 1045 mg/kg-day for female rats. Daily observations were made of mortality, clinical signs, and water consumption. Body weight, food consumption, and food utilization efficiency were measured weekly. A gross, post-mortem examination was performed at the end of the study. Organ weights (adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, tested, thyroid, and uterus) were measured and histopathological examinations of the liver and kidneys were performed.

Dose-related decreases in body weight gain were observed in both sexes, but the decrease was statistically significant (24% lower than controls particularly during the initial week of feeding) only in high-dose males. Food consumption in 10,000 ppm males was significantly lower (20%) than controls, suggesting that decreased body weight gain in high-dose males may have resulted from an unpalatable diet. No significant changes in food utilization were reported. Hepatic effects were observed in both sexes. Liver weights (adjusted using body weight as a covariate) were significantly increased in males at 10,000 ppm (24%) and in females at 1000, 5000, and 10,000 ppm (16-17%), but not at 3500 ppm. Gross hepatomegaly increased with dose in male rats, as shown in Table 1. The trend was found to be significant using the Cochran-Armitage test, while the incidence in the high-dose group was found to be significantly higher than controls using Fisher's exact test (Table 1; tests performed for this review). No significant differences were observed in females. Histopathology revealed minimal centrilobular hepatocyte enlargement in 4/5 males at 10,000 ppm, which, for this review, was determined to be significantly higher than controls using Fisher's exact test. Increase in liver weight and gross hepatomegaly are consistent with an adaptive response to the high dose of bifenox and are not by

themselves considered to be adverse. No statistically significant renal effects were observed grossly or histologically. In this study, a free-standing NOAEL of 10,000 ppm (904 mg/kg-day) was identified.

**Table 1. Incidence of liver effects in male Sprague-Dawley rats given dietary bifenox for 4 weeks (Huntingdon Research, 1984)**

| Effect | Exposure Level (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 35000 | 5000 | 10,000 |
| Gross hepatomegaly [a] | 0/5 | 0/5 | 1/5 | 2/5 | 2/5 | 5/5 [b] |
| Centrilobular hepatocyte enlargement | 0/5 | ND | ND | 0/5 | 0/5 | 4/5 [b] |

[a] Significant ($p<0.05$) using Cochran Armitage trend test performed for this review
[b] Significant ($p<0.05$) using Fisher's exact test performed for this review

Huntingdon Research (1987a) exposed Sprague-Dawley rats to bifenox in the diet for two years. Four groups of rats (50/sex/group) were fed diets containing 0, 500, 1580, or 5000 ppm of bifenox technical (purity 98%) for 104 weeks. Satellite groups of 20 rats/sex/dose were also included for blood sampling at intervals and for sacrifice at 52 weeks. Observed values for body weight and food consumption were used by the study authors to estimate mean doses of 0, 19, 59 or 188 mg/kg-day for male rats and 0, 25, 77 or 252 mg/kg-day for female rats. During the study, rats were checked twice daily for mortality. Observations for clinical signs were made daily for 4 weeks, and then, in the absence of any clinical reaction to that point, weekly for the remainder of the study. Body weights and food and water consumption were measured weekly, from which food utilization efficiencies were calculated. Ophthalmoscopy was performed on 10 rats/sex/group at weeks 0, 52, and 102. Blood samples were taken from 10 rats/sex/group at weeks 13, 27, 52 (again at week 53 for females, due to sampling problems on week 52), 77 and 103. Overnight urine samples were also collected from 10 rats/sex/group on weeks 14, 28, 52, 76, and 104. Hematological tests included packed cell volume (PCV), Hgb, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), counts of RBCs, total and differential white blood cells (WBCs), and platelets, thrombin time, and gross cell morphology. Measured serum biochemistry parameters included levels of glucose, alkaline phosphatase (ALP), aspartate-aminotransferase serum (AST) (reported by study authors as serum glutamic-oxaloacetic transaminase), alanine-aminotransferase (ALT) (reported by study authors as serum glutamic-pyruvic transaminase), albumin, globulin, total protein, blood urea nitrogen (BUN)**,** creatinine, sodium, potassium, calcium, inorganic phosphorus, chloride, and cholesterol. Urinalysis parameters included urine volume, pH, specific gravity, protein concentration, and qualitative measures of total reducing substances, glucose, ketones, bile pigments, urobilinogen, and heme pigments. Urine samples were also observed microscopically for presence of epithelial cells, mono- and polymorphonuclear leukocytes, erythrocytes, organisms, renal tubule casts, sperm, and other abnormal constituents. At study termination of 52 (for satellite groups) and 104 weeks, surviving rats were euthanized and given gross post-mortem examination of 37 tissue types. Adrenals, brain, heart, liver, kidneys, ovaries, pituitary, testes, and thyroid were weighed. These and 22 other tissues, plus all nodules, tissue masses, and grossly abnormal tissues, were examined for histopathology.

There were no dose-related trends in survival or clinical signs. Body weight gain and food consumption were significantly reduced over the first 26 weeks of the study in the high-

dose males, but subsequently recovered so that no differences from controls were found for the study as a whole. There were no statistically significant findings in organ weight, hematology, serum chemistry, urinalysis, or histopathology. Tumor incidence exhibited no statistical significance or dose-related trend. In this study, a NOAEL of 5000 ppm (188 and 252 mg/kg-day in males and females, respectively), was identified for chronic non-neoplastic effects. This study is limited for use in assessing cancer effects, as the lack of adverse effects at all doses indicates that the MTD was not achieved.

Charles River strain albino rats were given bifenox in the diet in a two year toxicity study (IBT, 1976a). Four groups of rats (60/sex/dose) were fed diets containing 0, 60, 200 or 600 ppm bifenox technical (purity not specified). For this review, values for body weight and food consumption from the study report were used to estimate doses of 0, 3, 10 or 31 mg/kg-day for male rats and 0, 4, 11 or 38 mg/kg-day for female rats. Daily observations were made for mortality and abnormal reactions. Body weights were measured weekly for the first 3 months of the study, and then monthly until study's end. Food consumption was measured and food utilization calculated weekly for 10 rats/sex/group for the first 3 months, and then monthly thereafter. Water consumption was measured on weeks 82-86 for 10 rats/sex/group. Blood samples were taken from 10 rats/sex from control and 600 ppm groups (or all survivors, if less than 10) at 3, 6, 12, and 24 months. Hematology measurements included counts of total and differential leukocytes, erythrocytes, and platelets, as wells as hematocrit and Hgb levels. Serum chemistry observations included ALP and ALT activities and glucose and BUN levels. Urine was analyzed for albumin and glucose levels, pH, specific gravity, and examination of microscopic elements. Upon unscheduled death, interim (10 rats/sex/group from the 0 and 600 ppm groups at 6 months of exposure) or terminal sacrifice, gross post-mortem exams were performed. Organ weights were recorded for liver, kidney, spleen, heart, and gonads. Histopathological exams of 27 different tissue types were conducted on all rats dying prematurely (unless precluded by severe autolysis) and up to 10 rats/sex/group of terminal survivors from the control and 600 ppm groups.

Mortality and clinical signs were similar across groups. Body weight gain was not adversely affected in any group. There were no significant differences between groups for food and water consumption. No statistically significant differences in hematology, serum chemistry, or urinalysis were observed between controls and 600 ppm rats. Similarly, no significant differences were observed in histopathological changes or neoplasm development between control and 600 ppm groups. However, histopathology was performed on only 4-13 males/group and 15-23 females/group of the possible 50 animals/group (not counting the 10 rats/group sacrificed at 6 months). The high dose of 600 ppm (31 and 38 mg/kg-day for males and females, respectively) was a NOAEL for non-neoplastic effects. This study is limited by the small number of animals examined for chronic histopathology and carcinogenesis, lack of reporting for compound purity, and as a cancer assay, failure to achieve a MTD.

A two-year oral toxicity and carcinogenicity study in $B6C3F_1$ mice was conducted by Litton Bionetics (1982). Four groups of mice (60/sex/dose) were fed diets containing 0, 50, 200 or 1000 ppm bifenox technical (purity 98.3%). Observed values for body weight and food consumption were used by the study authors to estimate monthly intake of bifenox. These monthly intakes were used in this assessment to estimate mean doses of 0, 7, 30 or 143 mg/kg-day for male mice and 0, 9, 35 or 179 mg/kg-day for female mice over the course of the study.

During the study, observations were made twice daily for mortality and weekly for clinical signs. Body weights were measured weekly for the first 12 weeks and biweekly thereafter. Food consumption was recorded every 4 weeks. Blood samples were collected from 10 mice/sex/group at 12 and 24 months. Hematology tests were performed, including measurement of hematocrit, Hgb levels, and RBC, platelet, reticulocyte, and total and differential leukocyte counts. Serum chemistry and urinalysis were not performed. At interim (12 months; 10 mice/sex/group) and terminal sacrifice, or following unscheduled death, liver, kidneys, brain, heart and testes were weighed following complete gross necropsy. Histopathological exams were performed on 30 tissue types, plus any other types exhibiting gross lesions at necropsy, from all animals from all dose groups. Histopathological exams were also performed on some additional tissues (the middle ear, paranasal sinuses, tongue, and oral and nasal cavities) from 10 rats/sex/group.

Mortality and clinical signs were unaffected by bifenox treatment. Body weights of all treated groups of males were significantly less than controls by 7-9% at 24 months, but not in a dose-related manner. Female body weights were not significantly different from controls. Changes in food consumption did not appear to be dose-related. Hematology results were unremarkable. In males, absolute, but not relative, brain, heart, and kidney weights were significantly higher than controls (3, 11, and 6%, respectively) in the 200 ppm group at 24 months, while 1000 ppm males exhibited a transient increase in absolute and relative liver weights (25% and 29%, respectively, at 12 months). These findings do not appear to be toxicologically significant. In females, kidney weights increased in a dose-related manner, with 24 month absolute and relative increases of 13 and 9%, respectively, observed in the 200 ppm group, and absolute and relative increases of 25 and 18% seen in the 1000 ppm group. Male mice given 50, 200, or 1000 ppm exhibited statistically significant renal histopathology: cytomegalic changes in renal tubule epithelium (focal hypertrophy, convoluted renal tubules), classified as "minimal to mild" by the study pathologist (Table 2). No significant renal histopathology was observed in females. No other significant, non-cancer histopathology was observed. A LOAEL of 50 ppm (7 mg/kg-day) was identified for renal focal hypertrophy of convoluted tubules in male mice. A NOAEL was not identified in this study.

**Table 2. Incidence of focal hypertrophy, convoluted renal tubules in B6C3F1 mice given dietary bifenox for 2 years (Litton Bionetics, 1982)**

| | **Exposure level (ppm)** | | | |
|---|---|---|---|---|
| | **0** | **50** | **200** | **1000** |
| Males [a] | 5/56 | 25/57[b] | 40/55[b] | 42/56[b] |
| Females | 0/50 | 0/53 | 2/54 | 4/55 |

[a] Significant ($p<0.05$) using Cox's trend test
[b] Significant ($p<0.05$) using Fisher's exact test performed for this review

The incidence of liver tumors (combined adenomas and carcinomas) was higher in males and females at 1000 ppm than in controls (Table 3). However, the researchers noted that the incidence of these tumors in treated animals was not unusually high for this age and strain of mouse. Statistical analyses performed by the researchers, adjusting for survival because the 12-month interim sacrifice animals were included in the tumor tabulation, showed no significant pairwise increase or trend in males, and only a trend of marginal significance (p=0.041) by one (Gehan-Breslow trend test) of three tests (including Cox's trend test and chi-square pair wise comparison) in females. The researchers did not consider these findings to represent evidence of oncogenicity.

**Table 3. Liver tumor incidence in B6C3F1 mice given dietary bifenox for 2 years (Litton Bionetics, 1982)**

| | Exposure level (ppm) | | | |
|---|---|---|---|---|
| **Tumor Type** | **0** | **50** | **200** | **1000** |
| *Males* | | | | |
| adenoma | 5/56 | 3/57 | 8/55 | 7/56 |
| carcinoma | 4/56 | 9/57 | 6/55 | 10/56 |
| combined adenoma and carcinoma | 9/56 | 11/57 | 14/55 | 17/56 |
| hemangiosarcoma | 4/56 | 1/57 | 0/55 | 0/56 |
| multifocal hemangiosarcoma | 0/56 | 0/57 | 0/55 | 1/56 |
| *Females* | | | | |
| adenoma | 1/52 | 3/58 | 0/56 | 3/55 |
| carcinoma | 1/52 | 0/58 | 0/56 | 2/55 |
| combined adenoma and carcinoma | 2/52 | 3/58 | 0/56 | 5/55 |
| hemangiosarcoma | 0/52 | 0/58 | 0/56 | 1/55 |
| metastatic hemangiosarcoma | 0/52 | 0/58 | 0/56 | 1/55 |

IBT (1976b) exposed purebred beagle dogs to dietary bifenox for two years. Four groups of dogs (6/sex/dose) were fed diets containing 0, 60, 200 or 600 ppm bifenox (purity not reported). Using reference values for food intake and body weight for beagle dogs (U.S. EPA, 1988), the doses are estimated as 0, 1, 4 or 12 mg/kg-day for both sexes. Daily observations were made for clinical signs. Weekly measurements were made of body weight and food consumption. Food utilization was calculated for the first 13 weeks of the study. At 3, 6, 12, 18, and 24 months, hematology, serum chemistry, and urinalysis were also performed. Hematologic tests included hematocrit, Hgb, MCV, MCHC, and counts of RBCs, total and differential leukocytes, and platelets. Serum chemistry measurements included levels of glucose, BUN, ALP, ALT, AST, cholesterol, and total protein. Urinalysis included observation of albumin and glucose levels, pH, specific gravity, and microscopic elements. At interim (2 dogs/sex/group at 6 months of exposure) and terminal sacrifice, gross post-mortem observations were made of all major tissues and organs. Organ weights were obtained for liver, lungs, kidney, heart, brain, spleen, gonads, and adrenal, thyroid, and pituitary glands. Histopathology was performed on 29

tissue types. Ophthalmoscopy was performed just prior to terminal sacrifice. No statistical analyses of study results were described or reported.

No mortality occurred and no unusual clinical signs were observed. Body weights, food consumption and utilization, and observed hematological, serum chemistry, and urinalysis parameters in treated dogs were deemed by the study authors to be comparable to controls and unrelated to treatment. Ophthalmoscopy was normal for all dogs. Observed pathological lesions were considered by the study pathologist to be normal disease occurrences and were not dose-related. Thus, a free-standing NOAEL of 600 ppm (12 mg/kg-day) was identified for beagle dogs.

Long-term toxicity of bifenox to beagle dogs was also studied by Huntingdon Research (1986), who administered capsule doses of bifenox technical (purity 98%) to four groups of beagle dogs (6/sex/group) for 52 weeks. Two dogs/sex/group were used for interim sacrifice at 26 weeks. Doses were 0 (empty capsule), 20, 145 or 1000 mg/kg-day. Daily observations were made of clinical signs and food consumption. Body weights were measured weekly, while ophthalmoscopy was performed and blood and urine samples collected on weeks 13, 26, and 52. Observed hematological parameters included Hgb, PCV, MCV, MCHC, and counts of RBCs, total and differential WBCs, platelets, and reticulocytes, RBC sedimentation rate, prothrombin and activated partial thromboplastin time, and cell morphology. Serum chemistry tests included measurements for levels of total protein, albumin, globulin, urea, creatinine, electrolytes, cholesterol, glucose, ALP, ALT, AST, ornithine carbamoyltransferase (OCT), gamma-glutamyltransferase (GGT), and total bilirubin. Urinalysis included observations of urine volume, pH, protein, total reducing substances, glucose, ketones, bile pigments, urobilinogen, heme pigments, and microscopic examination. Prior to interim (26 weeks) and terminal sacrifice, bone marrow obtained via sternal puncture was examined for cellularity, morphology, and cell distribution. Gross post-mortem exams were performed. Tissue weights were measured for adrenals, brain, heart, kidneys, liver, lungs, pancreas, pituitary, spleen, testes or ovaries, thymus, thyroids, and uterine or prostate. Histopathological examinations were performed on 36 tissue types.

No mortality or treatment-related clinical signs were observed. There were no significant differences between groups in body weights and gains, food consumption, or ophthalmoscopy. RBC levels in males given 1000 mg/kg-day were significantly lower (15%) than controls at week 26 only, but were within the range normally expected for dogs of this age. Serum calcium levels for all treated groups were significantly lower (4 to 7%) than controls at week 26. Males given 1000 mg/kg-day exhibited lower serum calcium levels before (4% lower) and throughout (up to 10% lower) the study. Calcium values for all groups, however, were reported to be within normal ranges. Males in the 1000 mg/kg-day group also had significantly ($p< 0.05$) elevated mean ALT (75% higher) and OCT (83% higher) levels at week 52, with values for 3 of 4 dogs exceeding the normal upper limits typically observed by the study authors (Table 4). Levels of ALT and OCT in females were not treatment related, with animals in the 1000 mg/kg-day group exhibiting week 52 ALT and OCT levels that were 14% higher and 4% lower, respectively, than controls. No treatment-related effects on urinalysis parameters were observed. Gross post-mortem examinations revealed no treatment-related effects. Terminal (52 week) liver weights, adjusted for body weight, were significantly higher in high-dose males (30%) and females (24%). Likewise, adjusted kidney weights were significantly higher in high-dose males (38%)

and females (23%). However, histological changes observed for these, and all other tissues, were not dose-related and were not useful for explaining relative weight increases for the liver and kidneys. A LOAEL of 1000 mg/kg-day, and an associated NOAEL of 145 mg/kg-day, were identified for significantly elevated serum levels of hepatic enzymes in male beagle dogs. Gross and histological observations found no neoplasms or dose-related incidence of pre-neoplastic lesions.

**Table 4. Mean (±SD) serum ALT and OCT levels (mU/ml) in beagle dogs given capsule doses of bifenox for 52 weeks (Huntingdon Research, 1986)**

| | **Dose level (mg/kg-day)** | | | |
|---|---|---|---|---|
| | **0** | **20** | **145** | **1000** |
| *males* | | | | |
| ALT | 32 ± 7.6 | 42 ± 15.9 | 36 ± 2.4 | 56 ± 18.7[a] |
| OCT | 5.9 ± 2.4 | 7.8 ± 1.0 | 7.6 ± 0.9 | 10.8 ± 3.7[a] |
| *females* | | | | |
| ALT | 29 ± 5.4 | 28 ± 3.1 | 26 ± 4.6 | 33 ± 5.6 |
| OCT | 7.2 ± 1.6 | 7.3 ± 1.7 | 7.0 ± 1.4 | 6.9 ± 1.0 |

[a] Significant ($p<0.05$) using William's test

IBT (1977) conducted a three-generation reproductive toxicity study in rats. Four groups of CD strain Charles River albino rats (10 males and 20 females per group) and their progeny were administered bifenox technical (purity 97.2%) in the diet at concentrations of 0, 20, 60 or 200 ppm. Using reference factors for food consumption and body weight in U.S. EPA (1988), the doses are estimated to be 0, 2, 6 or 20 mg/kg-day for males and 0, 2, 7 or 23 mg/kg-day for females. $F_0$ mating sets (1 male and 2 females) from each dose group were allowed to produce 2 litters, the first of which were sacrificed and discarded at weaning (day 21 post-partum) while the second were pooled with other litters within dose group to select $F_1$ parents (10 males and 20 females per group). This was repeated for $F_2$ progeny/parents. The study was terminated with the sacrifice of the $F_3$ progeny at weaning. Exposure duration comprised a pre-mating period (100 days), up to 3 attempts of 1$^{st}$ round of conception (up to 30 days total), gestation (21 days), a mating break period, if first mating was successful (10 days), 2$^{nd}$ round of conception (up to 30 days total), 2$^{nd}$ gestation (21 days), and pup rearing to weaning (21 days), for a total of up to 233 days for $F_0$, $F_1$, and $F_2$ parents. Daily observations were made for mortality, clinical signs, and abnormal behavior; however, body weights were taken weekly. Fecundity, male and female fertility, gestation length, lactation performance, survival, and live birth indices were also measured. Gross post-mortem examinations were performed on animals dying early. However, necropsies were not performed on all pups and adults; 8 males and females from pooled litters of each group were examined for gross pathology and organ weight. In addition, 5 males and

females from the control and 200 ppm groups, as well as all animals exhibiting grossly visible pathology at necropsy, were subjected to complete microscopic pathology exams.

No significant differences in mortality or clinical observations were found. Significantly higher paternal body weights (up to 24%) were observed in the 200 ppm group, compared to controls, in weeks 4 to 11, but not at termination; the study authors reported that the increased body weights were within the range normally observed in this strain of rat. Statistically significant changes in organ weight for liver, spleen, and gonads observed in 60 and 200 ppm parents were seemingly random for generation and organ type. $F_1$ and $F_2$ males given 200 ppm had 16 and 17% higher gonad weights, respectively, than controls. $F_2$ females given 200 ppm had 24% lower absolute liver weights than controls, while $F_2$ males and females given 60 ppm had 15 and 21% lower relative liver weights, respectively. $F_0$ males given 60 ppm and $F_1$ males given 200 ppm had 43 and 55% higher spleen weights, respectively, while relative weights were 32% and 21% higher for $F_0$ and $F_1$ males, respectively, given 200 ppm. These organ weight changes were not considered treatment-related. No significant differences in histopathology were observed. Reduced pup survival at weaning (postnatal day 21) was reported in the high-dose group of the $F_3$ generation. In one pooled litter of $F_3$ progeny (second mating) from the 200 ppm dose group, viable pups surviving to day 21 was 12% (12/98), or 1.0 pups/dam, compared with 57% (40/70), or 5.7 pups/dam for controls. However, survival indices were not different from controls for the pooled $F_3$ litter from the first mating of the 200 ppm group. Nor were effects on viability observed in any of the $F_1$ or $F_2$ litter groups at 200 ppm, or in any of the 20 and 60 ppm litter groups of any generation. Therefore, the reduced viability of pups in second mating $F_3$ litters at 200 ppm was considered not to be treatment-related. The high dose of 200 ppm (20 and 27 mg/kg-day for males and females, respectively) was identified as a NOAEL for reproductive effects in rats.

CDPR (1988) summarized a rabbit teratology study conducted by Hazleton Labs (1979). Bifenox (purity 98.3%) was administered by gavage in corn oil to groups of 15 artificially inseminated New Zealand White rabbits at doses of 0, 12.5, 25 or 50 mg/kg-day on gestational days (GD) 6-19. Maternal mortality and clinical signs (depression, prostration, tremors, fecal and urine stains, nasal or eye discharge, soft feces, wheezing, cyanosis, ataxia, dyspnea and hunched appearance) were reported in all dose groups. No developmental effects were reported at any dose. On the basis of the available study summary, there is not enough information available to adequately identify a maternal or developmental NOAEL or LOAEL.

In a second rabbit teratology study conducted by Hazleton Labs (1986), bifenox technical (purity 97%) was administered by gavage in 0.5% carboxymethylcellulose to artificially inseminated New Zealand White rabbits (16/group) at doses of 0, 5, 50, 160, 500 and 1000 mg/kg-day on GD 6-19. Observations were made during gestation for mortality, clinical signs, body weight, and food consumption. In the event of signs of abortion or premature delivery, does were euthanized and subjected to post-mortem examination, with additional observations made for implantation sites and resorbed and abnormally developing fetuses. Does surviving to term were sacrificed and subjected to post-mortem gross examination. Fetuses were examined viscerally for gross pathology and skeletal abnormalities. Gross pathologic examination of the fetuses demonstrated no abnormalies of viscera and musculo-skeletal systems.

Maternal mortality incidence was 14/16 (88% mortality by GD 25) in the 500 mg/kg-day group and 16/16 (100% mortality by GD 22) in the 1000 mg/kg-day group. Transient, but significant, body weight decreases of as much as 23% were observed in the 500 mg/kg-day group on GD 11-20. Food consumption in this group was 50-85% less than controls on GD 5-20 and 24-29. Likewise, body weights for the 1000 mg/kg-day group were decreased by as much as 41% on GD 8-20, while food consumption was 88% lower than controls on GD 6-8 and 100% less on GD 9-20. Clinical signs observed after treatment of ≥500 mg/kg-day included hypoactivity, ashen or pale appearance, body tremors and ataxia. The only unusual finding from gross pathological observation was hair and/or compound-like material found in the stomachs of prematurely dying does. Fetal effects could not be evaluated in the highest dose group because of complete maternal mortality. No dose-related developmental effects, including spontaneous abortion, were noted in the other treatment groups. In this rabbit study, a maternal FEL of 500 mg/kg-day, with an associated NOAEL of 160 mg/kg-day, was identified for increases in mortality and clinical signs in does. The developmental NOAEL was 500 mg/kg-day; due to maternal mortality, the 1000 mg/kg-day dose was not evaluated for developmental toxicity. This study found no developmental effects of bifenox in rabbits, even at dose levels highly lethal to the does.

A developmental toxicity study in rats was conducted by IBT (1972). In this study, mated albino rats (17-18/group) were administered bifenox technical (purity 99%) by gavage in corn oil at doses of 0, 50 and 100 mg/kg-day on GD 6-15. Dams were observed daily for mortality and clinical signs. Body weights were measured on GD 6, 9, 12, 15, and 20. At sacrifice on GD 20, observations were made for fetal swellings, implantation sites, uterine abnormalities, number of corpora lutea, viable fetuses, fetal weight, sex ratio, skeletal abnormalities, and gross internal and external abnormalities. No mortality or clinical signs were observed in dams, and maternal body weights were comparable throughout the study. Maternal uterine abnormalities were not found in any group. No treatment-related differences were found for any fetal parameters. The highest dose of 100 mg/kg-day was a NOAEL for both maternal and developmental toxicity.

CDPR (1988) summarized a rat teratogenicity study conducted by Huntingdon Research (1981a). Mated Sprague-Dawley rats (12/group) were administered bifenox (purity assumed to be 100%) by gavage in 1% methylcellulose at doses of 0 or 100 mg/kg-day on gestational GD 6 to postpartum day (PD) 21. A slight, but not statistically-significant decrease in relative and absolute liver weights was observed in treated dams, but no developmental toxicity was observed. In the absence of methodology description and data from the study, insufficient information was available to derive a NOAEL or LOAEL.

CDPR (1988) summarized a second rat teratogenicity study conducted by Huntingdon Research (1981b) in which mated Sprague-Dawley rats (24/group) were given diets containing 0, 500 or 1000 ppm of bifenox on GD 6 to PD 21. Using the reference body weight and the equations for food consumption (U.S. EPA, 1988), the doses are calculated as 0, 10, and 20 mg/kg-day. No significant effects were observed in dams or offspring. As with the prior rat study (Huntingdon Research, 1981a), the absence of actual study method descriptions and data precluded the derivation of a NOAEL or LOAEL.

A third rat teratogenicity study was conducted by Huntingdon Research (1987b) in which bifenox technical (purity 98%) was administered by gavage in 1% aqueous methylcellulose to mated Sprague-Dawley rats (25/group) at doses of 0, 225, 900 and 3600 mg/kg-day on GD 6-15. Animals were observed daily for mortality, clinical signs, and food consumption. Observations of water consumption began on GD 13. Body weights were measured on GD 1, 3, 6, 10, 14, 17, and 20. At GD 20, dams were sacrificed and internal organs examined for gross pathology. Ovaries and uteri were examined for number of corpora lutea, number and distribution of live and dead fetuses, fetal weight, and fetal external, internal, and skeletal abnormalities.

One dam from the 3600 mg/kg-day group died on GD 12 and 3 others were sacrificed *in extremis* on GD 8, 13, or 14. Post-mortem observations suggested that at least 3 of the 4 cases may have resulted from difficulties in dosing the highly viscous suspension. Occasional salivation, clear ocular discharge, lethargy, hunched posture, and patchy hair loss were seen in dams given 3600 mg/kg-day. While food consumption was transiently decreased in dams given 3600 mg/kg-day (10% lower than controls on GD 6-10), body weights were comparable across groups. Water consumption was also similar across groups. No significant developmental effects were observed at any dose. Thus, a maternal FEL of 3600 mg/kg-day, with an associated NOAEL of 900 mg/kg-day, was identified for increased mortality and clinical signs of toxicity in dams. The highest dose, 3600 mg/kg-day, was identified as a developmental NOAEL. Therefore, this study found that bifenox did not produce developmental effects in rats even at doses highly toxic to the dams.

Francis (1986) conducted a developmental study in Sprague-Dawley rats in which two groups of pregnant rats (6-10 per group) were given 0 or 100 mg/kg of bifenox by gavage in corn oil on GD 9, 10, 11 or 12. Litters were raised to weaning and observed daily for mortality. All pups were examined for the appearance of 'bloody tears,' a sign of inhibited Harderian gland development or respiratory disease. Bifenox had no significant effect on pups. The incidence of 'bloody tears' was 0% in control litters and 2.6% (1 pup) in treated pups on GD 10. Thus, a NOAEL of 100 mg/kg-day was identified for developmental toxicity in rats. There was insufficient data to identify a maternal NOAEL or LOAEL. Of note, rats exposed to nitrofen, a structural analog of bifenox, on GD 10 exhibited no statistically significant effect on pup survival; however, all litters exhibited 'bloody tears', resulting in an overall pup incidence rate of 67.7%.

In another experiment reported in the same paper, Francis (1986) gave gavage doses of 0, 10 or 100 mg/kg-day of bifenox (purity >99%) in corn oil to pregnant Swiss mice (7-13 per group) on GD 5-14. At termination on GD 18, observations were made of the number of live and resorbed fetuses and external fetal malformations. Changes in maternal body weight gain were not reported. No significant differences were seen in the number of litters per mated female or the number of live or resorbed fetuses per litter. Two exencephalic pups were found in a single bifenox-treated litter; however, the associated dose level was not reported. This defect did not occur in controls. Results of a later study (Francis et al., 1999) suggest that the exencephaly observed in this study was not related to treatment. Therefore, the high dose of 100 mg/kg-day was identified as a NOAEL for developmental toxicity in mice. The study did not provide enough information to identify a maternal NOAEL or LOAEL.

Francis et al. (1999) compared the maternal and developmental toxicity of eleven halogenated 4'-nitrodiphenyl ethers in mice. Mated female CD-1 mice were treated with 0, 250, 500, 750 or 1000 mg/kg-day of bifenox by gavage in corn oil on GD 6-15. The number of mated dams given bifenox, in order of increasing dose, was 6, 7, 2, and 12, though the rationale for these group sizes was not reported. One hundred fifty-three mated dams were in the control group. Dams were evaluated for clinical signs, weight gain between GD 6-16, mortality, number of litters born per group, the number of pups per mated female. Mean pup weights were reported for each group for PD 1, 5, and 15. At weaning (PD 30), 3 male and 3 female pups from each litter (except for the small 750 mg/kg-day group) were individually examined for body weight and the weight of the Harderian glands; regression analysis was used to evaluate dose effects on Harderian gland weight using sex, age and body weight as covariables.

The authors did not report mortality incidence. Bifenox had no significant effect on weight gain in dams that carried litters to term; weight gain results were not reported for dams given 750 mg/kg-day. The authors reported that for the diphenyl ethers in general, an increase in clinical signs (hunched posture, ruffled fur and vaginal bleeding) was correlated with a reduction in the number of litters born to treated females. The inference from prenatal mortality data provided for bifenox (Table 3 in the study report) is that these effects were observed in dams treated with bifenox at or above 750 mg/kg-day. Increases in prenatal losses of entire litters were dose-related; the incidence of prenatal litter loss was 10/153 in controls and 0/6, 1/7, 2/2 and 9/12 for the groups exposed to the lowest-to-highest doses of bifenox, respectively. For this review, statistical analysis of the incidence of litters lost prior to birth found a significant trend using the Cochran-Armitage test, and significant differences between controls and the 750 and 1000 ppm groups using Fisher's exact test. Bifenox had no statistically significant effect on the postnatal survival or body weight of litters, although both parameters were slightly reduced at the highest dose. In individually examined pups, the weight of the Harderian gland was slightly lower at the highest dose, but the difference was not statistically different from controls. This study is limited by its reporting deficiencies, necessitating extrapolation from the general comments on the toxicity of the tested diphenyl ethers. On this basis, the maternal and developmental NOAELs were 500 mg/kg-day and the LOAELs were 750 mg/kg-day for prenatal litter loss and the (presumed) increase in clinical signs associated with prenatal litter loss. Bifenox appears not to be a specific developmental toxicant because adverse effects were observed at maternally toxic doses and no significant postnatal effects were noted in litters surviving to term.

*Inhalation Exposure*

An acute inhalation LC50 of greater than 200 mg/L was reported for technical grade (96%) bifenox in rats (U.S.D.A., 1987). No relevant data were located regarding the toxicity of bifenox to animals following subchronic or chronic inhalation exposure.

**Other Studies**

Acute oral LD50 values for technical grade bifenox (purity >96%) were reported to be greater than 5000 or 6400 mg/kg for rats (U.S.D.A., 1987) and 4556 mg/kg for mice (RTECS). Eli Lilly & Co. (1981a) reported no mortality in mice receiving single oral doses as high as 9700 mg/kg. Differences between studies in lethality may be related to choice of dose vehicle. While 10% acacia was used as the vehicle in the Eli Lilly & Co. studies (1981a,b,c), the vehicles

associated with the LD50 values in U.S.D.A. (1987) and RTECS were not reported. No toxicity was observed in mice receiving 500 mg/kg, but doses between 700 and 9700 mg/kg caused generalized weakness of the limbs (Eli Lilly & Co., 1981a,b,c). The effect was transient, dissipating by the second hour after dosing in mice dosed with 700 or 1000 mg/kg and by the second day in mice dosed with 2750-9700 mg/kg.

Topical application of bifenox to mouse dams during gestation (Francis, 1986) resulted in effects similar to those seen in the oral mouse developmental study (Francis et al., 1999). Topical doses of 0, 1, 2 or 5 mg of bifenox dissolved in xylene were applied to the back skin of pregnant Swiss mice on GD 5-14; daily doses were reported as 0, 30-45, 60-90 or 150-200 mg/kg-day, respectively. The study authors reported that an apparent decrease in survival of bifenox-treated mice resulted from the complete loss of one litter in the highest dose group. Bifenox had no statistically significant effect on pup weight, the number of live pups at birth or at weaning, or the weight of the Harderian gland in pups. In parallel groups, nitrofen treatment at the highest dose significantly increased pup mortality, and reduced the mean weight of pups from postnatal day 1 to weaning, as well as the weights of the Harderian glands and eyes.

Several investigators used in vitro assays to evaluate the ability of bifenox to induce mutagenicity, genotoxicity, and endocrine disruption. *In vitro* genotoxicity studies on bifenox reported generally negative results, with or without metabolic activation (unless otherwise noted, all studies reviewed here used both test conditions). Bifenox was not mutagenic to *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, or TA100 (American Biogenics Corp., 1986; Hokky Kagaku Kogyo Laboratory, 1982; EG&G Mason Research Institute, 1979, as described in CDPR, 1988; Eisenbeis et al., 1981; Kubo et al. 2002), *Escherichia coli* strains WP2 hcr (U.S.D.A, 1987) or WP2 uvrA (Hokky Kagaku Kogyo Laboratory, 1982 as described in CDPR, 1988), or to *Saccharomyces cerevisiae* (Plewa et al., 1984). In tests with two strains of *S. typhimurium* that contain high nitroreductase activity, bifenox was not mutagenic to YG1021, but was, with activation, slightly mutagenic in YG1026 (3 revertants per mg) (Oguri et al., 1995). Mutagenicity was also seen in *S. typhimurium* strains YG1024 and 1026 (with metabolic activation), but not in YG1021, 1029, 3003, and 7108, with or without metabolic activation (Tanaka et al., 2002). Bifenox was not mutagenic in Chinese hamster ovary cells *in vitro* (Pharmakon Res. Intl., as described in CDPR, 1988). No increase in chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with bifenox (American Biogenics Corp., as described in CDPR, 1988) or in cultured bovine peripheral lymphocytes treated with bifenox as the commercial herbicide Modown (Sivikova and Dianovsky, 1999), although dose-related increases in the sister chromatid exchange and decreases in mitotic and proliferation indices were exhibited, with significant reductions occurring at the highest concentrations (250-1000 μg/ml). Without metabolic activation, bifenox did not induce unscheduled DNA synthesis in cultured primary rat hepatocytes (Litton Bionetics, as described in CDPR, 1988). Bifenox gave negative results in a cell transformation assay using C3H/10T0T1/2 cells without metabolic activation (EG & G Mason Res. Inst., as described in CDPR, 1988).

*In vivo*, bifenox was not clastogenic to bone marrow of male Sprague-Dawley rats that received single gavage doses as high as 1500 mg/kg (Mobil Environ. Health Sci. Lab., as described in CDPR, 1988). In mice that received two intraperitoneal injections of bifenox 24 hours apart, no evidence of micronucleus formation was observed in males at doses as high as 1440 mg/kg/injection, whereas an increase in micronucleus formation was observed in females at

doses of 480 or 720 mg/kg/injection (1440 mg/kg was lethal to females) (Borriston Labs, Inc., as described in CDPR, 1988).

In a screening of pesticides to test for androgenic receptor activity using an in vitro reporter gene in Chinese hamster ovary cells, Kojima et al. (2004) found bifenox to be negative for estrogenic activation via the human estrogenic receptor α and β genes, but positive for inhibition of human androgenic receptor transcriptional activity when induced by 5α-dihydrotestosterone. The authors suggest that this may implicate bifenox as an endocrine disruptor.

## DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR BIFENOX

No human data are available to derive a provisional subchronic or chronic RfD for bifenox. Animal studies describe the effects of bifenox administered orally in the diet or as bolus doses to mice, rats, dogs, and rabbits. Kidney and liver effects appear to be the most sensitive endpoints (Table 5). Rats exposed to 904 mg/kg-day in the diet for 4 weeks (Huntingdon Research, 1984) exhibited significant increases in liver weights and liver histopathology (centrilobular hepatocyte enlargement); however, these are considered adaptive responses to chemical exposure rather than toxic effects. Beagle dogs given 1000 mg/kg-day by capsule for 1 year (Huntingdon Research, 1986) exhibited significantly increased blood levels of ALT (75% higher than controls) and OCT (83% higher than controls), indicative of liver toxicity, as well as increased liver and kidney weights. Two-year dietary exposure studies in rats (IBT, 1976a; Huntingdon Research, 1987a), dogs (Huntingdon Research, 1987a), and mice (Litton Bionetics, 1982) did not report adverse liver effects; however, exposure levels reported in these studies were notably lower than 1000 mg/kg-day (252 mg/kg-day in female rats in Huntingdon Research, 1987a was the maximum dietary level). Kidney effects were observed at chronic doses as low as 7 mg/kg-day in mice, including renal histopathology (renal tubule cytomegalic changes without progression to hyperplasia and necrosis) in males and, at the higher doses, increased kidney weights in females.

A number of developmental toxicity studies in rats, mice, and rabbits suggest that the neonate is less sensitive than the mother to bifenox toxicity (Table 6). Gestational exposure of rats (Francis, 1986; IBT, 1972) and mice (Francis, 1986) to gavage doses of 100 mg/kg-day did not result in maternal (results not reported in all studies) or developmental toxicity. Frank maternal effects, including mortality, were observed in rabbits given 500 or 1000 mg/kg-day by gavage from GD 6-19 (Hazelton Labs, 1986), rats given 3600 mg/kg-day from GD 6-15 (Huntingdon Research, 1987b), and are presumed to have occurred in mice given 750 or 1000 mg/kg-day from GD 6-15 (Francis et al., 1999), although poor reporting of maternal toxicity in this study makes interpretation uncertain. No fetal effects occurred in the rat or rabbit studies. Full litter loss was observed in the mouse study, but this may reflect a maternal, rather than fetal effect. In any event, the increase in full litter loss was only seen at maternally toxic doses. A 3-generation reproduction study in rats given bifenox in the diet (IBT, 1977) found reduced 21-day survival of $F_3$ pups of the pooled litter from the second mating in the high-dose group given 20 mg/kg-day. However, no effect on pup viability was seen in the corresponding $F_3$ pooled litter

**Table 5. Non-cancer effects and effect levels identified from studies of oral (dietary and capsule dosing) bifenox exposure to animals**

| Source | Species | Exposure duration / sub-route | NOAEL mg/kg-day | LOAEL mg/kg-day | Effect |
|---|---|---|---|---|---|
| Huntingdon Research, 1984 | rats | 4 weeks, dietary | 904 | ND | adaptive increases in liver weight and centrilobular hepatocyte enlargement |
| IBT, 1977 | rats | up to 233 days (multigeneration), dietary | 20 | ND | no dose-related findings |
| IBT, 1976a | rats | 2 years, dietary | 31 | ND | no dose-related findings |
| Huntingdon Research, 1987a | rats | 2 years, dietary | 188 | ND | no dose-related findings |
| Litton Bionetics, 1982 | mice | 2 years, dietary | ND | 7 | renal tubule cytomegalic changes in males |
| Huntingdon Research, 1986 | dogs | 1 year, capsule | 145 | 1000 | increased serum ALT and OCT levels in males |
| IBT, 1976b | dogs | 2 years, dietary | 12 | ND | no dose-related findings |

ND = Not determined

**Table 6. Non-cancer effects and effect levels identified from developmental studies of oral gavage exposure to animals**

| Source | Species | Exposure duration / sub-route | NOAEL mg/kg-day | LOAEL mg/kg-day | Effect |
|---|---|---|---|---|---|
| Francis et al., 1999 | mice | GD 6-15, gavage | maternal: 500<br>fetal: 500 | maternal: 750<br>fetal: 750 | clinical signs (presumed) in dams; prenatal litter loss |
| Francis, 1986 | mice | GD 5-14, gavage | maternal: ND<br>fetal: 100 | maternal: ND<br>fetal: ND | no dose-related fetal findings; inadequate reporting of maternal effects |
| Huntingdon Research, 1987b | rats | GD 6-15, gavage | maternal: 900<br>fetal: 3600 | maternal: 3600<br>fetal: ND | maternal mortality (FEL) and clinical signs; no fetal effects |
| Francis, 1986 | rats | GD 9,10,11 or 12, gavage | maternal: ND<br>fetal: 100 | maternal: ND<br>fetal: ND | no dose-related fetal findings; inadequate reporting of maternal effects |
| IBT, 1972 | rats | GD 6-15, gavage | maternal: 100<br>fetal: 100 | maternal: ND<br>fetal: ND | no dose-related maternal or fetal findings |
| Hazleton Labs, 1986 | rabbit | GD 6-19, gavage | maternal: 160<br>fetal: 500 | maternal: 500<br>fetal: ND | maternal mortality (FEL), clinical signs, and reductions in food consumption and body weight; no fetal effects |

GD = gestation day , ND = Not determined, FEL = frank effect level

group from the first mating, or in the $F_1$ or $F_2$ generations at this same dose, or in the lower dose groups. Therefore, this effect was considered not to be treatment-related (Table 5).

*Subchronic RfD*

Studies considered in derivation of the subchronic RfD include the 4-week study in rats, the one-year study in dogs (considered a subchronic duration for this species, since one year is approximately 10% of a lifetime in dogs), and the reproductive and developmental studies. As discussed above, the developmental studies demonstrated that the fetus is not a sensitive target for bifenox independent of maternal toxicity. The frank maternal toxicity seen in these studies was probably a by-product, at least in part, of the bolus dosing used in these studies. Frank effects were accompanied by observations of compound-like material in the stomach (Hazelton Labs, 1986), which may be a result of gavage dosing of bifenox suspension. The dietary administration used in the systemic toxicity and multigeneration studies more closely

corresponds to the expected human environmental exposures than the bolus dosing used in the developmental studies. Among the dietary studies, only the one-year dog study identified a critical effect and effective levels. Effects in the multigeneration study (IBT, 1977) were considered not to be treatment-related, while those in the 4–week rat study (Huntingdon Research, 1984) were not clearly adverse.

The 52-week dietary exposure study in beagle dogs (Huntingdon Research, 1986) was selected as the principal study for derivation of a subchronic RfD since it identified a LOAEL of 1000 mg/kg-day, with an associated NOAEL of 145 mg/kg-day, based on clear, treatment-related elevations of the liver enzymes ALT and OCT in serum of male beagle dogs. Benchmark dose analysis, using the Benchmark Dose Modeling Software (BMDS), version 1.3.2, was performed on the ALT and OCT data from the male dogs to estimate doses (BMD) and lower bounds on the 95% confidence interval (BMDL) associated with a benchmark response (BMR) of 1 standard deviation from controls, as recommended by U.S. EPA (2000) for continuous data. However, the variances across dose groups were not constant and the power model built into the BMDS was not able to adequately fit the variance data for either data set. Because the data could not be modeled satisfactorily, derivation of the subchronic provisional RfD is based on the observed LOAEL of 1000 mg/kg-day and NOAEL of 145 mg/kg-day for significant increases in the serum levels of the liver enzymes ALT and OCT in male beagle dogs.

The provisional subchronic RfD of 1 mg/kg-day is derived by dividing the NOAEL of 145 mg/kg-day for increased serum levels of hepatic enzymes in males identified in the 52-week capsule dosing study in dogs (Huntingdon Research, 1986) by a composite uncertainty factor (UF) of 100, as follows:

| | | |
|---|---|---|
| Subchronic p-RfD | = | 145 / UF |
| | = | 145 mg/kg-day / 100 |
| | = | **1 mg/kg-day** |

The composite UF includes a factor of 10 for extrapolation from animals to humans and 10 for inter-individual variability. No uncertainty factor for database deficiencies was applied due to the relatively complete database for the chemical, including a multigeneration reproduction study and developmental toxicity studies in multiple species.

Confidence in the critical study is medium. The study investigated an adequate array of endpoints, but group sizes were small. Dose-related increases in serum levels of two liver enzymes in male dogs provided reasonable evidence of hepatotoxicity. However, the magnitude of the observed changes was somewhat less than the 2-3 fold increase conventionally considered to represent clinical significance for these effects, and no corresponding histopathological lesions were detected. Further, comparable changes were not found in females. Confidence in the database is medium-to-high. Extensive clinical and histopathology data were available from well-conducted studies in rats and dogs, but several studies did not include dose levels high enough to observe effects. In addition to systemic toxicity studies, the database includes developmental toxicity studies in rats, mice and rabbits and a multigeneration reproduction study in rats. Overall confidence in the provisional subchronic RfD is medium.

*Chronic RfD*

Studies of toxicity from chronic bifenox exposure have been performed in mice, rats, and dogs. The studies in rats and dogs failed to find any effects at dietary doses up to 188 mg/kg-day in rats (IBT, 1976a; Huntingdon Research, 1987a) and 12 mg/kg-day in dogs (IBT, 1976b). The mouse study (Litton Bionetics, 1982) found renal effects at 7 mg/kg-day and above in males (minimal to mild focal hypertrophy of convoluted renal tubules) and at 35 mg/kg-day and above in females (increased absolute and relative kidney weights). Minimal to mild focal hypertrophy of convoluted renal tubules was also occasionally noted in female mice. The incidence of renal histopathology in male mice is clearly dose-related and a NOAEL was not identified (Table 2). The only other report of renal effects in the literature was an increase in kidney weight in the one-year dog study at a dose of 1000 mg/kg-day, with no effect at 145 mg/kg-day (Huntingdon Research, 1986). Developmental and reproductive effects have been shown not to be sensitive endpoints for bifenox, as discussed above.

Based on these data, mice appear to be the most sensitive species, and the kidney the most sensitive endpoint with chronic dietary exposure. The 2-year mouse study of Litton Bionetics (1982) was chosen as the principal study for derivation of a chronic RfD because it identified the lowest LOAEL of 7 mg/kg-day in the diet for histopathological renal changes over the lifespan of the most sensitive test species. A NOAEL was not identified. Dose-response modeling was performed using the BMDS (Version 1.3.2) and a benchmark response (BMR) of 10% extra risk. All available dichotomous models were fit to the data and the best fit was determined using guidelines described in U.S. EPA (2000). The results of the BMDS modeling are summarized in Appendix A. None of the models produced adequate fits using data from all four exposure levels. By dropping the highest exposure level, as described in U.S. EPA (2000), an adequate fit was achieved by the log-logistic model, with estimated values of 1.3 and 0.9 mg/kg-day for the $BMD_{10}$ and $BMDL_{10}$, respectively.

The provisional chronic RfD of 0.009 mg/kg-day is derived by dividing the $BMDL_{10}$ of 0.9 mg/kg-day for renal effects identified in a 2-year dietary exposure study in mice (Litton Bionetics, 1982) by a composite UF of 100 as follows:

| | | |
|---|---|---|
| Chronic p-RfD | = | $BMDL_{10}$ / UF |
| | = | 0.9 mg/kg-day / 100 |
| | = | **0.009 or 9E-3 mg/kg-day** |

The composite UF includes a factor of 10 for extrapolation from animals to humans and 10 for inter-individual variability. No uncertainty factor for database deficiencies was applied due to the relatively complete database for the chemical, including a multigeneration reproduction study and developmental toxicity studies in multiple species.

Confidence in the principal study is medium. Although the study was well-conducted with a sufficient number of animals, the critical effect, a clear, dose-related increase in renal histopathology, was observed in males only and a NOAEL was not identified. Females showed an increase in kidney weight, but no renal lesions, and only at higher doses. However, it should be pointed out that minimal to mild focal hypertrophy of convoluted renal tubules was also occasionally noted in female mice. Confidence in the database is medium-to-high. Extensive

clinical and histopathology data were available from well-conducted studies in mice, rats and dogs, but several studies did not include dose levels high enough to observe effects. In addition to systemic toxicity studies, the database includes developmental toxicity studies in rats, mice and rabbits and a multigeneration reproduction study in rats. Overall confidence in the provisional chronic RfD is medium.

## FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR BIFENOX

No human or animal data are available to derive a provisional subchronic or chronic RfC for bifenox.

## PROVISIONAL CARCINOGENICITY ASSESSMENT FOR BIFENOX

### Weight-of-evidence Classification

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), bifenox is classified as having *inadequate information to assess carcinogenic potential*. Lifetime studies of bifenox in the diet of Sprague-Dawley (Huntingdon Research, 1987a) and an unspecified strain of albino (IBT, 1976a) rats found no significant increases in incidence of tumor development in either strain. However, an MTD was not reached in either study. No carcinogenic effects were observed in beagle dogs given capsule (Huntingdon Research, 1986) or dietary (IBT, 1976b) administrations of bifenox for 1 and 2 years, respectively. The data from the dog studies are of limited utility for carcinogenicity assessment, however, as group sizes were small (6/sex/dose, with 2/sex/dose sacrificed at 6 months) and exposures were considerably less than lifetime (1-2 years). Small apparent increases in liver tumors (combined adenoma and carcinoma) in male and female B6C3F1 mice treated with bifenox in the diet for two years (Litton Bionetics, 1982) were within the historical control range and either not statistically significant (males) or only marginally significant ($p=0.041$) by one (Gehan-Breslow trend test) of three tests (including Cox's trend test and chi-square pair wise comparison) (females). The researchers did not consider these findings to represent evidence of oncogenicity. Assays for genotoxicity of bifenox were primarily negative, including in vitro and in vivo assays for mutagenicity, clastogenicity, DNA effects, and cell transformation.

### Quantitative Estimates of Carcinogenic Risk

Since the data for bifenox are inadequate to assess carcinogenic potential, no quantitative estimates for carcinogenic risk from oral or inhalation exposures are derived.

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# APPENDIX A

Dose-response modeling using the BMDS (Version 1.3.2) and a BMR of 10% extra risk was performed on the incidence data of Litton Bionetics (1982) for cytomegalic renal hypertrophy of convoluted renal tubules of male B6C3F1 mice. Modeling produced estimates ($BMD_{10}$) and lower bounds on the 95% confidence interval ($BMDL_{10}$) of exposure levels likely to result in 10% extra risk of developing cytomegalic renal hypertrophy in mice. All available dichotomous models were fit to the data and the best fit was determined using guidelines described in U.S. EPA (2000). Goodness-of-fit was evaluated using the Chi-square statistic calculated by the BMDS. Adequate model fit to the data was indicated by a p-value $\geq 0.1$; models with a p-value $< 0.1$ were not considered. Subsequently, $BMDL_{10}$ estimates were ranked using the Akaike Information Criterion (AIC) reported by the BMDS program. The model with the lowest AIC was considered to provide a superior fit. Modeling results are summarized in Table A-1. No adequately-fit models resulted from use of all four dose levels; dropping the high dose resulted in adequate fit of a single model, the log-logistic model (Figure A-1). Fit of the log-logistic model resulted in $BMD_{10}$ and $BMDL_{10}$ estimates of 1.34 mg/kg-day and 0.91 mg/kg-day, respectively.

**Table A-1. Benchmark dose modeling results for cytomegalic changes (focal hypertrophy) of convoluted renal tubules in male B6C3F1 mice (Litton Bionetics, 1982) – high dose dropped**

| Model | AIC | p-Value | $BMD_{10}$ | $BMDL_{10}$ |
|---|---|---|---|---|
| Quantal Linear | 182.974 | 0.0984 | 2.3162 | 1.7827 |
| Weibull[a] | 182.974 | 0.0984 | 2.3162 | 1.7827 |
| Multistage[b] | 182.974 | 0.0984 | 2.3162 | 1.7827 |
| Gamma[a] | 182.974 | 0.0984 | 2.3162 | 1.7827 |
| Log Probit[c] | 185.351 | 0.0210 | 3.8078 | 2.9072 |
| Log Logistic[c] | **180.398** | **0.7671** | **1.3366** | **0.9113** |
| Logistic | 189.814 | 0.0026 | 5.2781 | 4.2860 |
| Probit | 189.479 | 0.0030 | 5.1003 | 4.2267 |
| Quantal Quadratic | 194.996 | 0.0002 | 9.0841 | 7.6675 |

Abbreviations: AIC = Akaike Information Criterion; $BMD_{10}$ = Benchmark Dose, maximum likelihood estimate of the dose producing a 10% extra risk in convolutes renal tubules; $BMDL_{10}$ = 95% lower confidence limit on the $BMD_{10}$.
[a]Power restricted to >=1
[b]Betas restricted to >=0, Degree of polynomial = 1
[c]Slope restricted to >=1

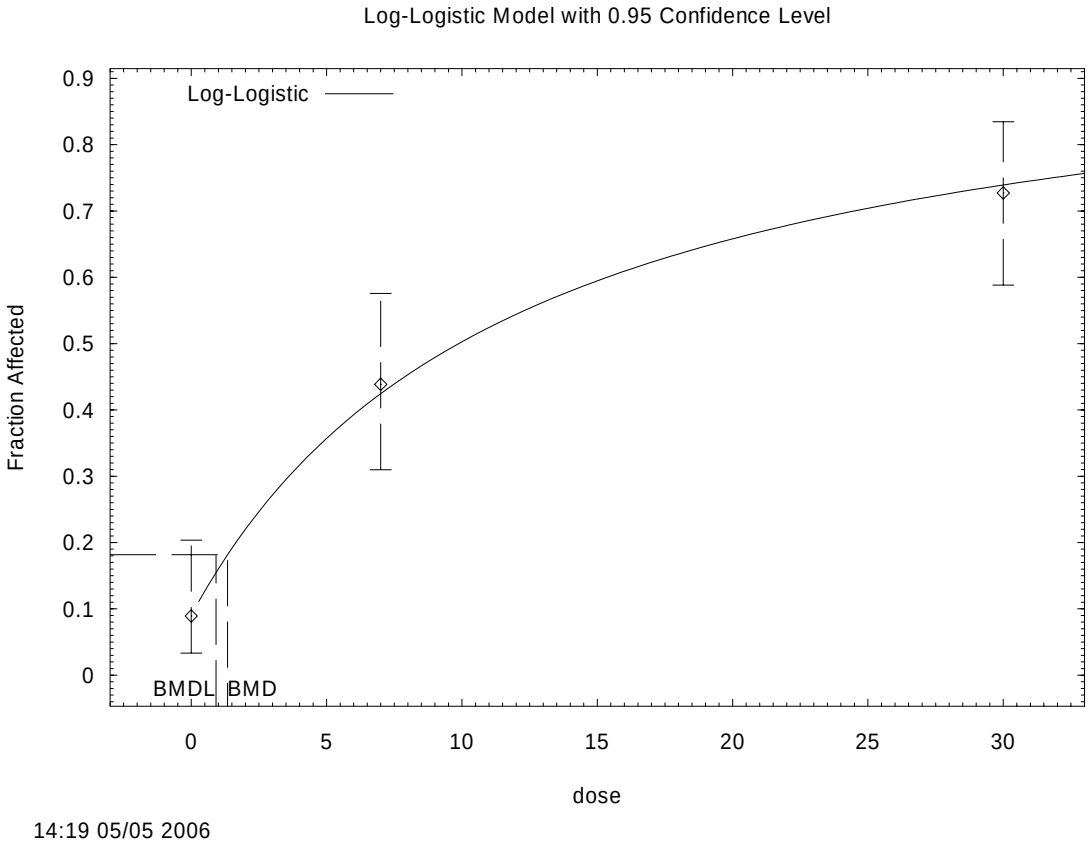


**Figure A-1. Fit of the Log-Logistic dose-response model to the incidence of focal hypertrophy of convoluted tubules in male B6C3F1 mice given dietary bifenox for 2 years (Litton Bionetics, 1982)**

```
 ================================================================
        Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
        Input Data File: C:\BMD\BIFENOX\LITTON.(d)
        Gnuplot Plotting File:  C:\BMD\BIFENOX\LITTON.plt
                                            Fri May 05 14:19:25 2006
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = incidence
   Independent variable = dose_ppm
   Slope parameter is restricted as slope >= 1

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0892857
                     intercept =     -2.48897
                         slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

             background    intercept

background            1        -0.37

 intercept        -0.37            1



                          Parameter Estimates

       Variable           Estimate            Std. Err.
     background          0.0906975           0.0384068
      intercept           -2.48738            0.239702
          slope                  1                  NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.
```

Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|---|---|---|---|---|
| Full model | -88.1553 | | | |
| Fitted model | -88.1989 | 0.0871849 | 1 | 0.7678 |
| Reduced model | -114.104 | 51.8984 | 2 | <.0001 |

AIC: 180.398

Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|---|---|---|---|---|---|
| 0.0000 | 0.0907 | 5.079 | 5 | 56 | -0.03679 |
| 7.0000 | 0.4252 | 24.235 | 25 | 57 | 0.2049 |
| 30.0000 | 0.7397 | 40.686 | 40 | 55 | -0.2107 |

Chi-square = 0.09 DF = 1 P-value = 0.7671

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.33664

BMDL = 0.911304