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

OFFICE OF PREVENTION,
PESTICIDES, AND TOXIC SUBSTANCES

TXR No.: 0054799

MEMORANDUM

DATE: January 4, 2008

SUBJECT: TRICLOSAN: Report of the Cancer Assessment Review Committee

PC Code: 054901

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509P)

Jessica Kidwell

TO: Tim McMahon, Toxicologist (IO)
Antimicrobials Division (7510P)

The Cancer Assessment Review Committee met on July 25, 2007 to evaluate the carcinogenic potential of Triclosan. Attached please find the Final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

TRICLOSAN

PC code 054901

FINAL
January 4, 2008

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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DATA PRESENTATION: Tim McMahon, Toxicologist

DOCUMENT PREPARATION: Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise noted.)

Gregory Akerman

Karlyn Bailey

Lori Brunsman, Statistician

William Burnam, Chair

Marion Copley

Kit Farwell

Ray Kent

Mary Manibusan

Jess Rowland

Yin-Tak Woo — See attached sheet

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist — See attached sheet

FINAL

DATA PRESENTATION: Tim McMahon, Toxicologist

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TRICLOSAN CANCER ASSESSMENT DOCUMENT FINAL

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John Pletcher, Consulting Pathologist

EXECUTIVE SUMMARY

On July 25, 2007, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Triclosan.

On March 10, 1998, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) examined the existing carcinogenicity data for Triclosan and was unable to assign a classification to Triclosan at that time since data for only one species (rat) were submitted for evaluation of carcinogenicity. Since then, additional data have become available to the Agency, specifically a chronic toxicity/carcinogenicity study in the hamster (MRID 44874001) and a carcinogenicity study in the mouse. The mouse study data were not submitted to the Agency as the study was conducted by Colgate-Palmolive for the Food and Drug Administration and Colgate-Palmolive did not agree to submit the study data. There is no legal requirement for them to do so since they are not the pesticide's registrant. However, the Agency has obtained the review of the mouse carcinogenicity study conducted by the Food and Drug Administration as well as additional documentation regarding the significance of the mouse study results for human health. Data on metabolism and mutagenicity were also available. Several studies supporting a proposed mode of action (MOA) involving peroxisome proliferation were also accessible. These data were presented to the CARC by Tim McMahon of the Antimicrobials Division.

After consideration of all the available data, the CARC reached the following conclusions:

Carcinogenicity

Rat

•No treatment-related increase in tumors was seen in male or female Sprague-Dawley rats at doses up to 3000 ppm.

•Adequacy of Dosing: The dose of 3000 ppm (168/217.4 mg/kg/day, M/F) in the rat chronic toxicity/carcinogenicity study was considered to be adequate and not excessive for purposes of carcinogenicity testing. Mortality was not adversely affected in this study. Mean body weight of male rats was significantly decreased only up to week six of the study (~6%) and mean body weight of female rats was decreased between 5-10% from weeks 3-76 of the study. Significantly increased incidences of non-neoplastic lesions of the liver in males (cytoplasmic inclusions and hepatocellular hypertrophy) were also observed at 3000 ppm as was an increased incidence of renal calculi at this dose.

Hamster

•No treatment-related increase in tumors was seen in male or female BioF1D Alexander Syrian hamsters at doses up to 250 mg/kg/day.

•Adequacy of Dosing: Dosing at the high dose of 250 mg/kg/day was considered adequate and not excessive. This was based on increased mortality in high dose males only after week 80, decreased body weight (84-85% and 89-90% of controls, males/females, respectively) and body weight gain in both sexes (46-53% of controls through week 90), hematological alterations (increased urea nitrogen, increased serum triglycerides), and non-neoplastic lesions of the kidney (distended medullary tubules, increased incidence and severity of nephropathy), increased incidence of stomach lesions, and partial depletion of one or more generation of germ cells in the testis.

Mouse

• In male CD-1 mice, the incidence of liver adenomas, carcinomas, and combined adenomas and/or carcinomas for the control, 10, 30, 100, and 200 mg/kg/day dose groups were as follows:

Adenomas:	5/50 (10%), 7/50 (14%), 13/50 (26%), 22/50 (44%), 26/49 (53%)
Carcinomas:	2/50 (4%), 3/50 (6%), 6/50 (12%), 11/50 (22%), 24/49 (49%)
Combined:	6/50 (12%), 10/50 (20%), 17/50 (34%), 32/50 (64%), 42/49 (86%)

Male rats had significant increasing trends at $p<0.005$ for adenomas, carcinomas, and combined adenomas and/or carcinomas. There were significant differences in the pair-wise comparisons of the 30, 100, and 200 mg/kg/day dose groups with the controls, for combined liver adenomas and/or carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 100 and 200 mg/kg/day dose groups with the controls, for liver adenomas and carcinomas, all at $p<0.01$. The incidence of adenomas and adenomas and/or carcinomas combined exceeded historical control incidences (10% for adenomas; 17% for combined) at the ≥10 mg/kg/day dose level, but were statistically significant at the ≥30 mg/kg/day dose level. The incidence of carcinomas exceeded the historical control incidence (7%) at ≥30 mg/kg/day, but was statistically significant at ≥100 mg/kg/day. The CARC considered the liver tumors in male rats to be treatment-related.

• In female CD-1 mice, the incidence of liver adenomas, carcinomas, and combined adenomas and/or carcinomas for the control, 10, 30, 100, and 200 mg/kg/day dose groups were as follows:

Adenomas:	0/50 (0%), 1/50 (2%), 3/50 (6%), 6/50 (12%), 11/50 (22%)
Carcinomas:	0/50 (0%), 0/50 (0%), 1/50 (2%), 1/50 (2%), 14/50 (28%)
Combined:	0/50 (0%), 1/50 (2%), 3/50 (6%), 6/50 (12%), 20/50 (40%)

Female rats had significant increasing trends at $p<0.005$ for adenomas, carcinomas, and combined adenomas and/or carcinomas. There were significant differences in the pair-wise comparisons of the 100 and 200 mg/kg/day dose groups with the controls, for liver adenomas and combined adenomas and/or carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 200 mg/kg/day dose groups with the controls, for liver carcinomas, at $p<0.01$. The incidence of adenomas and adenomas/carcinomas combined exceeded historical control incidences (1% for adenomas; 1% for combined) at the ≥10 mg/kg/day dose level, but were statistically significant at the ≥100 mg/kg/day dose level. The incidence of carcinomas exceeded the historical control incidence (0%) at ≥30 mg/kg/day, but was statistically significant at 200 mg/kg/day. The CARC considered the liver tumors in female rats to be treatment-related.

• Adequacy of Dosing: The dose level of 200 mg/kg/day was considered adequate in both sexes and not excessive for carcinogenicity testing. At ≥30 mg/kg/day there was a dose-related increase in liver weight and hepatocellular hypertrophy. In addition, increased mortality was observed in male mice at 100 mg/kg/day and in female mice at 200 mg/kg/day.

Mutagenicity

• Triclosan has intrinsic mutagenic activity *in vitro* but this is not expressed in whole animals. Accordingly there is no mutagenicity concern for triclosan.

Structure Activity Relationship (SAR)

• No appropriate analogs were available for comparison.

Mode of Action

• There is sufficient weight-of-the-evidence to establish a mode of action for peroxisome proliferator-activated receptor alpha (PPARα) for triclosan-induced hepatocarcinogenesis in mice. Key precursor events and the tumor response in mice were concordant with respect to both time and dose. While the proposed mode of action for liver tumors in mice is theoretically plausible in humans, it is quantitatively implausible and unlikely to take place when quantitative species differences in PPARα activation and toxicokinetic and toxicodynamic factors are taken into account (i.e. the formation of liver tumors in mice has no relevance to humans) (Klaunig et al 2003). With regards to alternative mode(s) of action, the data did not support either a mutagenic mode of action or cytotoxicity followed by a sustained cellular regenerative response.

Classification and Quantification of Carcinogenic Potential

In accordance with the EPA Final Guidelines for Carcinogen Risk Assessment (March 29, 2005), the CARC classified Triclosan as "Not Likely to be Carcinogenic to Humans". This decision is based on the weight of evidence that supports activation of peroxisome proliferator activated receptor alpha (PPARα) as the primary mode of action for triclosan-induced hepatocarcinogenesis in mice. The data did not support either a mutagenic mode of action or cytotoxic mode of action that is consistent with a sustained regenerative cellular proliferative response. While the proposed mode of action for liver tumors in mice is theoretically plausible in humans based on the availability of a functional PPAR-alpha receptor, hepatocarcinogenesis via this mode of action is quantitatively implausible and unlikely to take place in humans based on quantitative species differences in PPARα activation and differences in toxicokinetics.

The quantification of risk is not required.

I. INTRODUCTION

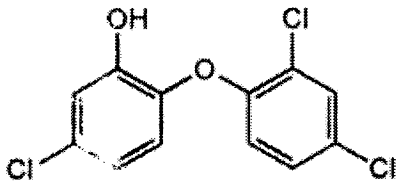
On July 25, 2007, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Triclosan.

On March 10, 1998, the Health Effects Division's HIARC committee examined the available carcinogenicity data for Triclosan and was unable to assign a classification to Triclosan at that time since data for only one species (rat) were submitted for evaluation of carcinogenicity. Since then, additional data have been made available to the Agency, specifically, a chronic toxicity/carcinogenicity study in the hamster (MRID 44874001), and a carcinogenicity study in the mouse. The mouse study data were not submitted to the Agency as the study was conducted by Colgate-Palmolive for the Food and Drug Administration and Colgate-Palmolive did not agree to submit the study data. There is no legal requirement for them to do so since they are not the pesticide's registrant. However, the Agency has obtained the review of the mouse carcinogenicity study conducted by the Food and Drug Administration as well as additional documentation regarding the significance of the mouse study results for human health. Data on metabolism and mutagenicity were also available. Several studies supporting a proposed mode of action (MOA) involving peroxisome proliferation were also available.

II. BACKGROUND INFORMATION

Triclosan (2,4,4'–trichloro-2'-hydroxydiphenyl ether) is a chlorinated aromatic compound that has functional groups representative of both phenols and ethers. It is used as a synthetic broad-spectrum antimicrobial agent in the form of a white to off-white powder. It is practically insoluble in water but is soluble in most organic solvents. Triclosan's uses include hundreds of common everyday products, such as soaps, deodorants, toothpastes, laundry detergents, fabric softeners, facial tissues, and diapers. Triclosan is also impregnated in products such as kitchen utensils, toys, bedding, socks, and trash bags.

Structure:



III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Reference: Yau, E. and Green, J. (1986): FAT 80'023: 2-year Oral Administration to Rats. Study conducted for Ciba-Geigy Dyestuffs and Chemical Division, Greensboro, NC by Ciba-Geigy Pharmaceuticals Division, Summit, NJ. (MRID 42027906).

A. Experimental Design:

In a chronic toxicity/carcinogenicity feeding study (MRID 42027906) conducted in male and female Sprague-Dawley rats, FAT 80'023 (triclosan, 99.0 % a.i.) was administered in the diet at doses of 0, 300, 1000, or 3000 ppm (0, 15.3, 52.4, and 168.0 mg/kg/day in males; 0, 20.0, 66.9, and 217.4 mg/kg/day in females) for 104 weeks. An additional group of 20 male and 20 female rats received triclosan in the diet at 6000 ppm (415.0 mg/kg/day [males] and 519.3 mg/kg/day [females]) for 52 weeks.

B. Discussion of Mortality and Tumor Data

No treatment related effects on mortality, were observed at any dose level tested. At 104 weeks, percent mortality was 63%, 70%, 53%, and 63% for males in the 15.3, 52.4, and 168.0 mg/kg/day dose groups respectively. In females, percent mortality was 67%, 68%, 65%, and 70% for the 20.0, 66.9, and 217.4 mg/kg/day dose groups respectively.

No carcinogenic potential was demonstrated for triclosan in this study.

C. Non-Neoplastic Lesions

A summary of non-neoplastic lesions observed in this study is shown in the following Table:

TABLE 14. Selected Nonneoplastic Lesion in Rats Fed Fat 88*023 for 104 Weeks

	Dose Level (ppm)									
	Males					Females				
Organ/Finding	0	300	1000	3000	6000[c]	0	300	1000	3000	6000[c]
Liver[a]	(95)[b]	(85)	(85)	(85)	(20)	(95)	(85)	(85)	(85)	(20)
Cytoplasmic inclusions of hepatocytes	0	0	0	4	4*[d]	0	0	0	0	0
Centrilobular hepatocyte hypertrophy	0	0	0	7	12**[d]	0	0	0	0	0
Cellular alteration	13	17	18	14	0	13	15	9	15	0
Hyperplasia of liver and bile duct	19	13	19	7	0	11	13	11	11	0
Necrosis	1	5	4	4	0	0	0	0	2	0
Telangiectasis	12	21*[e]	24**[e]	16	0	0	2	6	1	0
Congestion	12	18	14	10	0	14	9	8	7	0
Vacuolation	15	3	10	19	9	16	12	9	12	0
Pancreas[f]	(80)			(78)	(20)	(80)			(70)	(20)
Focal atrophy of acinar tissue	19	9[g]	13[g]	18	2	13	4[g]	5[g]	10	5
Hyperplasia of pancreatic islets	3	1[g]	1[g]	4	5	1	0[g]	0[g]	0	0
Kidney[f]	(80)	(68)	(60)	(60)	(20)	(79)	(60)	(60)	(68)	(20)
Microscopic renal calculi	3	5	9	12**[e]	0	19	21	19	9	0
Mineralization	1	3	4	5	0	8	10	20**[e]	18	0

(continued)

TABLE 14. Continued

Organ/Finding	Dose Level (ppm)									
	Males					Females				
	0	300	1000	3000	6000[c]	0	300	1000	3000	6000
Lung[f]	(80)	(60)	(60)	(60)	(20)	(80)	(60)	(60)	(60)	(20)
Accumulation of foamy macrophages (alveoli)	15	29**e	22	26*e	0	6	16*e	19**e	14*e	0

[a]Includes animals at the 13-, 26-, and 78-week serial sacrifices, at the 52-week interim sacrifice, at terminal sacrifice, and those that died or were sacrificed moribund during the study.

[b]Number in parentheses equals number of tissues examined.

[c]All high-dose animals were sacrificed at 52 weeks.

[e]Significant effect at week 52 as evaluated by the study authors.

[e]Significant effect at week 104 as evaluated by the study authors.

[f]Includes animals at the 52-week interim sacrifice, at the terminal sacrifice, and those that died or were sacrificed moribund during the study.

[g]Finding observed in nonroutine organs; only animals with finding were examined histologically.

*Significantly different from controls at $p < 0.05$ as evaluated by the study authors.

**Significantly different from controls at $p < 0.01$ as evaluated by the study authors.

***Significantly different from controls at $p < 0.001$ as evaluated by the study authors.

The incidence of cytoplasmic inclusions and centrilobular hepatocyte hypertrophy in males was found to be significantly increased at the 168 and 415 mg/kg/day dose levels. The review noted that these increases were noted in males at 13, 26, and 78 weeks at the 168 mg/kg/day dose level and noted at 52 weeks at the 415 mg/kg/day dose level based on serial sacrifice data. Increases in these liver lesions were not noted in female rats. The incidence of hepatic necrosis and telangiectasis was found to be increased in male rats at the 15.3, 52.4, and 168.0 mg/kg/day dose levels, but the incidence did not show a dose-response and was not considered treatment related. Increases in these liver lesions were not observed in female rats.

The incidence of renal calculi was found to be significantly increased in male rats at the 168 mg/kg/day dose level and appeared dose-related, but a similar finding was not observed in female rats.

In a memorandum from Dr. Lucas Brennecke to Dr. Esther Rinde dated June 6, 1997, the issue of the significance of the hepatocellular necrosis observed in the rat study was addressed. Dr. Timothy McMahon requested Dr. Brennecke to consider the conclusions of a report submitted by Ciba-Geigy in which a Pathology Working Group had convened to resolve discrepancies arising from interpretation of the hepatic necrosis observed in the rat study. Dr. Brennecke's conclusion was that the necrosis observed in this study (classified as zonal necrosis) should be considered as an incidental finding in this study.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The dose of 3000 ppm in the rat chronic toxicity/carcinogenicity study was considered to be adequate and not excessive for purposes of carcinogenicity testing. Mortality was not adversely affected in this study. Mean body weight of male rats was significantly decreased only up to week six of the study (~6%) and mean body weight of female rats was decreased between 5-10% from weeks 3-76 of the study. Significantly increased incidence of non-neoplastic lesions of the liver in males (cytoplasmic inclusions and hepatocellular hypertrophy) were also observed at 3000 ppm as was an increased incidence of renal calculi at this dose.

2. Chronic Toxicity/Carcinogenicity Study In Hamsters

Reference: Chambers P.R. 1999. FAT 80'023/S (Irgasan® DP300R): Potential tumorigenic and chronic toxicity effects in prolonged dietary administration to hamsters. Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Laboratory report number, CBG 756/972896, March 30, 1999. MRID 44874001. Unpublished.

A. Experimental Design

In a chronic toxicity/carcinogenicity study (MRID 44874001), FAT 80'023/S (99.5% a.i.; Batch # 505017) was administered in the diet to groups of 70 male and 70 female Bio F1D Alexander Syrian hamsters at concentrations delivering doses of 0 (control 1), 0 (control 2), 12.5, 75, or 250 mg/kg/day. Groups of 10 hamsters per sex per dose were killed after 52 weeks for interim evaluations; the remaining 60 hamsters per sex per dose were maintained on treated or control diets for up 90 weeks for females and 95 weeks for males.

B. Discussion of Mortality and Tumor Data

No treatment-related clinical signs of toxicity were observed during the first 80 weeks of the study. After this time, high-dose males showed a deterioration in their general clinical condition with signs such as lethargy, hunched posture, pallor, thin appearance, and unsteady gait. At termination of the females (week 91) the percent survival in the control 1, control 2, low-, mid-, and high-dose groups was 40%, 38%, 47%, 58%, and 48%, respectively. In contrast, high-dose males had an increase in mortality after week 80 which correlated with their deteriorating clinical condition. At termination of the males (week 96) the percent survival in the control 1, control 2, low-, mid-, and high-dose groups was 65%, 72%, 75%, 80%, and 35%, respectively.

No evidence of potential carcinogenicity of the test material was observed at the doses given in this study. Neoplastic lesions did not occur in treated groups at incidences significantly higher than the incidences in control animals.

C. Non-neoplastic Lesions

At interim sacrifice, irregular cortical scarring of the kidney was observed at gross necropsy in 4/10 high-dose males and 9/10 high-dose females compared with none in the control male groups and 3/19 in the control female groups combined. This corresponded to microscopic findings in the kidneys of the high-dose groups of both sexes consisting of distended medullary tubules and radial areas of dilated basophilic tubules with or without eosinophilic colloid/fibrosis.

At terminal sacrifice, no dose- or treatment-related gross findings were observed in males. However, in the control (combined), low-, mid-, and high-dose female groups,

white nodules in the forestomach were observed in 3/46, 3/28, 5/35, and 5/29 animals, respectively, pale kidneys were observed in 14/46, 4/28, 3/35, and 10/29 animals, respectively, and irregular cortical scarring was observed in 24/46, 12/28, 16/35, and 20/29 animals, respectively. Microscopically, a significantly ($p \leq 0.01$) increased incidence of nephropathy was observed in high-dose males and females (decedents and survivors combined) as compared to both control groups and was considered the main factor contributing to death in animals that died before study termination. The severity of nephropathy, as calculated by the reviewer, in high-dose males and females was 3.2 and 2.8, respectively, compared with control values of 2.5-2.7 and 2.1-2.3, respectively. The incidence of nephropathy in the control 1, control 2, low-, mid-, and high-dose groups was 41/60, 38/60, 35/60, 36/60, and 56/60, respectively, for males and 19/60, 21/60, 26/60, 19/60, and 50/60, respectively, for females.

A summary of non-neoplastic lesions from the hamster study is shown below in Table 1:

Table 1. Notable histopathologic findings in hamsters fed FAT 80'023/S					
Organ/lesion	**Dose (mg/kg/day)**				
	0	**0**	**12.5**	**75**	**250**
Interim					
Kidney, distended medullary tubules					
Males	1/10	2/10	1/9	1/9	9**##/10
Females	8/10	5/9	5/10	6/10	10/10
Kidney, Radial areas of dilated basophilic tubules +/- eosinophilic colloid/fibrosis					
Males	1/10	0/10	0/9	0/9	8**##/10
Females	3/10	4/9	2/10	0/10	9**#/10
Terminal (decedents + survivors)[a]					
Nephropathy					
Males	41/60	38/60	35/60	36/60	56**##/60
Females	19/60	21/60	26/60	19/60	50**##/60
Stomach, focal atypical hyperplasia - fundic region					
Males	0/60	0/60	0/60	0/60	11**##/60
Females	0/60	0/60	0/60	0/60	1/60
Stomach, distended gastric glands +/- debris					
Males	6/60	9/60	16/60	10/60	10/60
Females	1/60	3/60	8/60	7/60	17**##/60
Epididymides					
Abnormal spermatogenic cells	3/60	2/60	4/60	3/60	20**##/60
Reduced numbers of spermatozoa	2/60	5/60	2/60	1/60	20**##/60
Spermatozoa absent	7/60	4/60	5/60	6/60	12/60#
Testes					
Partial depletion of one or more generations of germ cells	12/60	12/60	12/60	10/60	40**##/60

Data taken from text tables pp. 39 and 41-43, and Table 13, pp. 148-150, MRID 44874001.
[a]Statistical analysis for combined incidence rates calculated by reviewer using Fisher's Exact test.
Significantly different from control group 1: *p≤0.05, **p≤0.01.
Significantly different from control group 2: #p≤0.05, ##p≤0.01.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing at the high dose of 250 mg/kg/day was considered adequate and not excessive. This was based on increased mortality in high dose males only after week 80, decreased body weight (84-85% and 89-90% of controls, males/females, respectively) and body weight gain in both sexes (46-53% of controls through week 90), hematological alterations (increased urea nitrogen, increased serum triglycerides), and non-neoplastic lesions of the kidney (distended medullary tubules, increased incidence and severity of nephropathy), increased incidence of stomach lesions, and partial depletion of one or more generation of germ cells in the testis.

3. Carcinogenicity Study in Mice

Reference: 18-month dietary carcinogenicity study in mice conducted for Colgate-Palmolive and reviewed by the Food and Drug Administration. Permission to cite this review was granted in a letter from Nancy Sager, Director, Division of Information Disclosure Policy, to Tim McMahon, Ph.D., dated June 20, 2007.

A. Experimental Design

In a carcinogenicity bioassay in mice submitted to the Food and Drug Administration, 5 groups of male and female CD-1 mice (70 mice/sex/dose) received triclosan in the diet at dose levels of 0, 10, 30, 100, or 200 mg/kg/day. Fifty mice/sex/dose received dietary triclosan for 18 months, while the remaining 20 mice/sex/dose received dietary triclosan for only 6 months, after which time these mice were sacrificed. Blood samples were obtained from 10 mice/sex/dose from both the 6 month and 18 month dose groups at sacrifice for determination of triclosan plasma levels. Time of blood sampling relative to the last dose of triclosan was not stated. Parameters monitored during this study included mortality, clinical observations, body weight, food consumption, ophthalmology, clinical chemistry, urinalysis, hematology, gross and microscopic pathology, and organ weights.

B. Discussion of Survival and Tumor Data

Survival

Reduced survival was observed in female mice receiving 200 mg/kg/day for 18 months (34/50 vs. 45/50 in control). Calculated survival for animals in this study (based on 50 animals/dose/sex at study initiation) were 88%, 86%, 82%, 64%, and 78% survival rate for males, and 90%, 86%, 88%, 84%, and 68% survival for females. The review considered that the reduced survival in males at the 100 mg/kg/day dose level 'may not have been completely related to treatment (since a higher survival rate was observed in males at 200 mg/kg./day)' 'However, the reduced survival of the high-dose females must be assumed to have been related to treatment.'

Tumors

After 18 months of exposure, a statistically significant increase in the incidence of hepatocellular adenoma and carcinoma was observed in male and female mice at 100 mg/kg/day triclosan and above. The incidence was dose-related in both sexes. Combined incidence of adenoma and carcinoma was 12%, 20%, 34%, 64%, and 86% for males, and 0%, 2%, 6%, 12%, and 40% for females at the 0, 10, 30, 100, and 200 mg/kg/day dose levels, respectively. The following data were available from the FDA review:

Table 5. Incidence of Liver Tumors in Mice Fed Triclosan

Dosage of Triclosan (mg/kg/day)	Number of Animals with Adenomas		Number of Animals with Carcinomas		Number of Animals with Adenomas and/or Carcinomas	
	Males	Females	Males	Females	Males	Females
0	5 (10%)	0 (0%)	2 (4%)	0 (0%)	6 (12%)	0 (0%)
10	7 (14%) p=0.2898	1 (2%) p=0.4717	3 (6%) p=0.3378	0 (0%) p=1.0000	(20%) p=0.1617	1 (2%) p=0.4717
30	13 (26%) p=0.0204	3 (6%) p=0.1112	6 (12%) p=0.0920	1 (2%) p=0.4835	17* (34%) p=0.0083	3 (6%) p=0.1112
100	22* (44%) p<0.0001	6* (12%) p=0.0093	11* (22%) p=0.0015	1 (2%) p=0.4615	32* (64%) p<0.0001	6* (12%) p=0.0093
200	26* (53%) p<0.0001	11* (22%) p<0.0001	24* (49%) p<0.0001	14* (28%) p<0.0001	42* (86%) p<0.0001	20* (40%) p<0.0001

*p<0.01 in pair-wise comparisons to control group.

Tests for trends toward an increased incidence of adenomas, carcinomas, or the combined incidence with increasing dose were significant at the p<0.005 level in both males and females. Histopathology was performed on liver samples from 50 animals per group per sex, except for males at 200mg/kg/day, of which only 49 animals were examined. Dosages of 10mg/kg/day and 30mg/kg/day were no effect levels (NOELs) in regard to tumorigenicity in males and females, respectively.

Historical control incidence of hepatocellular neoplasms were also presented as part of the FDA review (source unavailable) and are copied from the review. The data are reproduced below:

Table 6. Historical control data (Hepatic Tumor Incidence)

Tumor	Males	Females
Adenoma	10%	1%
Carcinoma	7%	0%
Adenoma or Carcinoma	17%	1%

According to the FDA review, male mouse data were from 11 studies that totaled 641 male mice, and female mouse data were from 10 studies that totaled 581 female mice. The dates of these studies were not noted in the review.

C. Non-Neoplastic Lesions

Data for non-neoplastic lesions observed in this study were not available for review except in graphical form from the December 2000 report entitled "Implications for Human Health of the Triclosan Animal Bioassay Data," by the Triclosan Expert Panel. Permission to cite this report was obtained from Ciba Specialty Chemicals. The summary of the review stated the following:

Mean liver weight (absolute and relative) was increased in both male and female mice at 30 mg/kg/day and above at both 6 and 18 months. An increased incidence of nodules and discoloration of the liver was observed in both male and female mice at 100 mg/kg/day and above. A dose-related increase in severity of hepatocellular hypertrophy was observed in both male and female mice at 30 mg/kg/day and above. Dose-related increases in incidence or severity of hepatocellular vacuolation/vesiculation and hepatic inflammation, necrosis, and microgranulomas was also observed.

The following graphical data were available:

FIGURE 3
Hepatocellular Necrosis in Mice Fed Triclosan

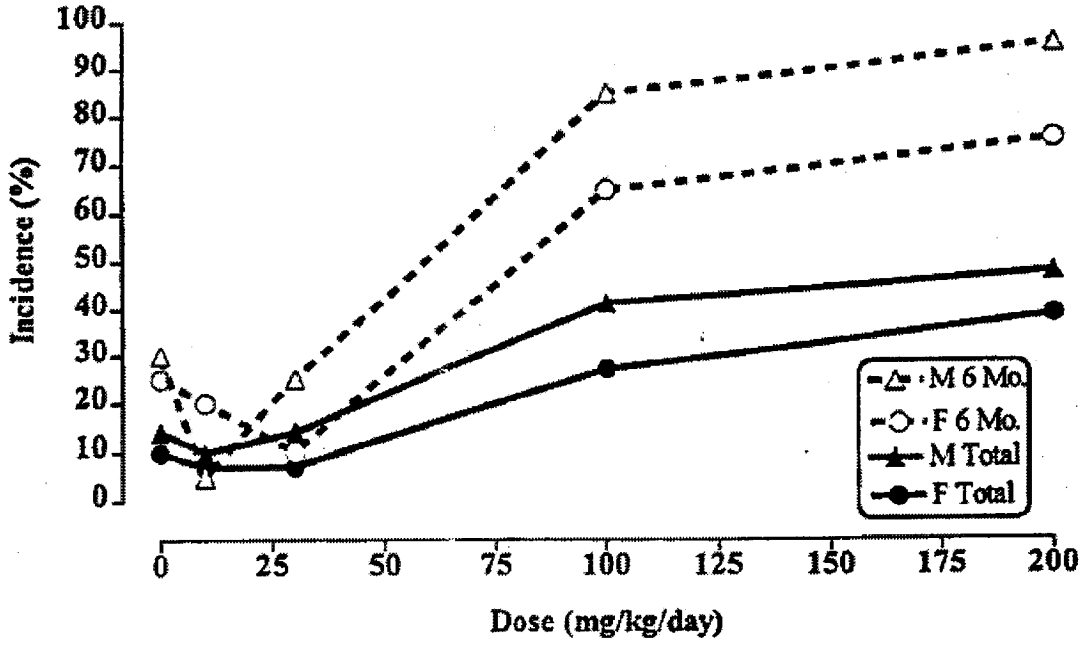


FIGURE 4
Liver "Brown Pigment" in Mice Fed Triclosan

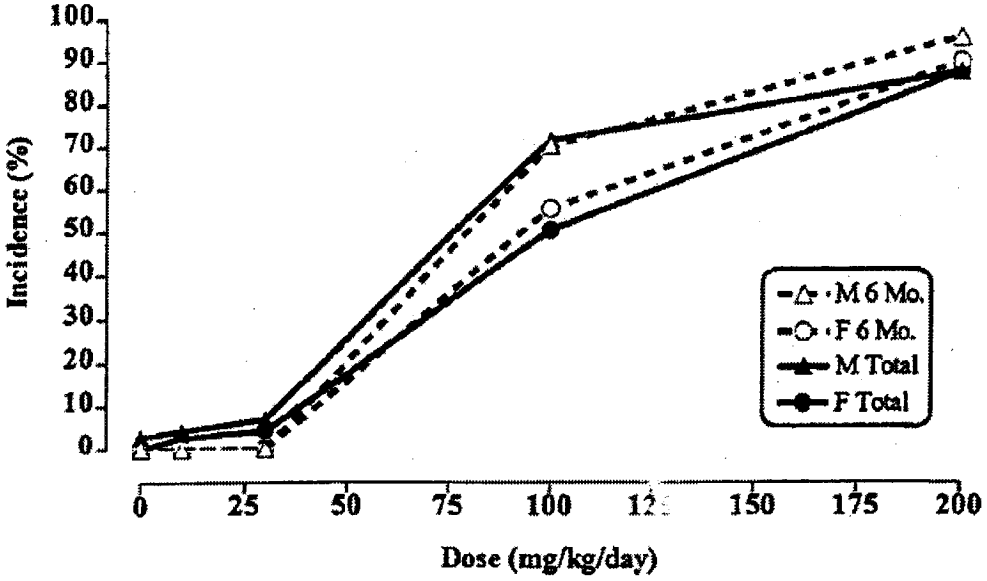


FIGURE 5
Hepatocellular Hypertrophy in Mice Fed Triclosan

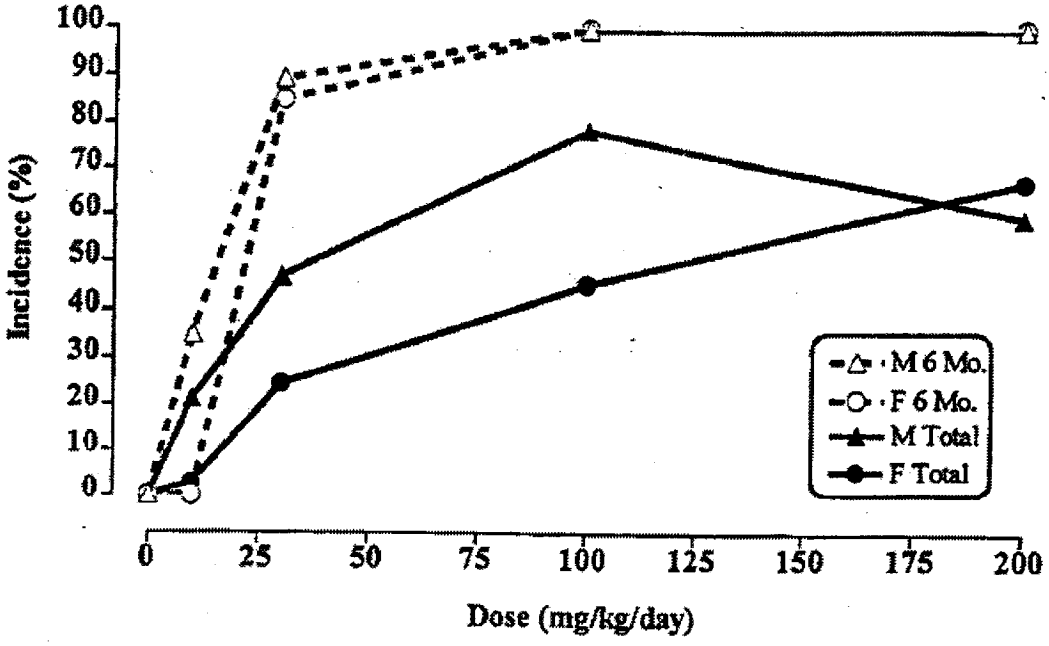
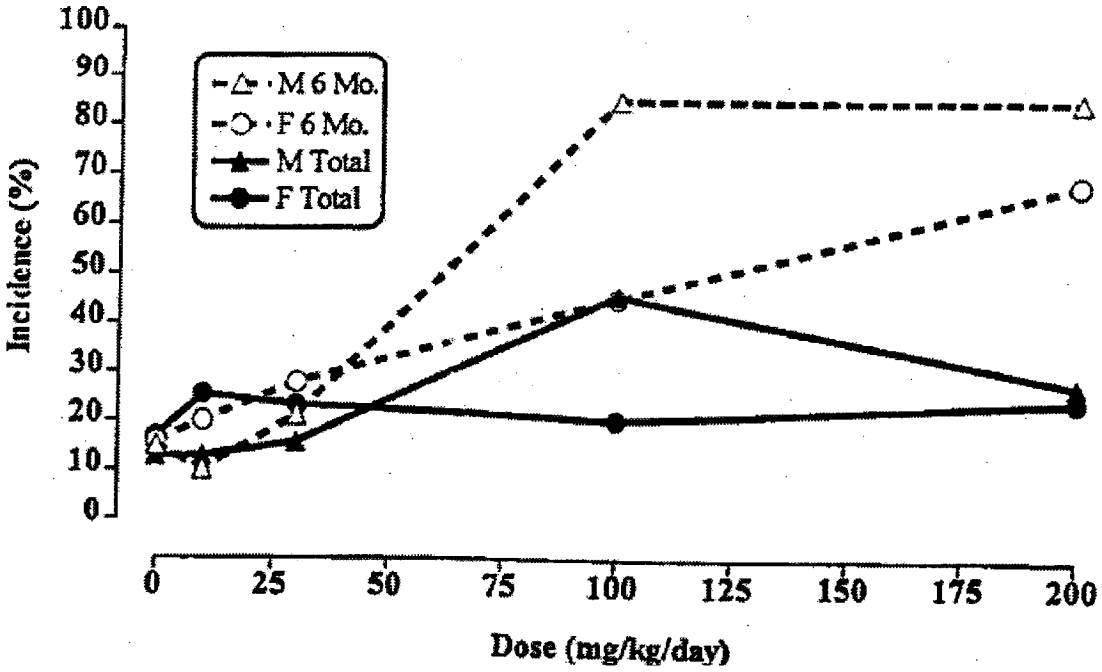


FIGURE 6
Liver Microgranulomas in Mice Fed Triclosan



D. Adequacy of Dosing for Assessment of Carcinogenicity

The dose level of 200 mg/kg/day was considered adequate and not excessive for carcinogenicity testing. At ≥30 mg/kg/day there was a dose-related increase in liver weight and hepatocellular hypertrophy. In addition, increased mortality was observed in male mice at 100 mg/kg/day and in female mice at 200 mg/kg/day.

IV. TOXICOLOGY

1. Metabolism

Several studies are available on metabolism and disposition of triclosan, including absorption, distribution, metabolism, and elimination studies in hamsters (MRID 45307501, 45307502), mice (MRID 45307503), mice, rats, rabbits, and dogs (MRID 149464), mice, rats, dogs, and baboons (MRID 68161), and dogs and baboons (MRID 79590).

The overall findings from the metabolism data in various species suggests that the general disposition of Triclosan is most similar between hamsters and humans. Both species show urinary excretion as the major route (mice and rats show greatest excretion in feces), and the glucuronide conjugate of Triclosan appears to be the major urinary metabolite in both humans and hamsters (the mouse shows both sulfate and glucuronide conjugates in urine, and free Triclosan in feces).

2. Mutagenicity

Triclosan has been tested for mutagenic activity in several assays, including bacterial reverse mutation tests (MRID 43533301 and MRID 44389705), an *in vitro* mammalian cell gene mutation test (MRID 44389704), two *in vitro* mammalian chromosome aberration tests (MRID 47276601 and MRID 43740801), a mammalian bone marrow chromosomal aberration test (MRID 43740802), and an unscheduled DNA synthesis assay in mammalian cells in culture (MRID 47076602).

A. Gene Mutations

In two independently performed microbial preincubation assays (MRID 43533301), *Salmonella typhimurium* strains TA1535, TA1537, TA98, or TA100 were exposed to triclosan doses of 0.015, 0.050, 0.15, 0.5, or 1.5 µg/plate either in the absence or the presence of 3, 10, or 30% S9 derived from Aroclor 1254-induced rat livers. The test material was delivered to the test system in dimethyl sulfoxide.

Triclosan was cytotoxic at 1.5 µg/plate-S9 and at doses ≥0.5 µg/plate with S9. **There was, however, no indication of a mutagenic response in any strain at any dose either without or with increasing concentrations of S9.**

In a microbial mutagenicity assay (MRID 44389705), Salmonella typhimurium strains TA100 and TA1538 were exposed to triclosan (100.5% a.i.) in dimethylsulfoxide (DMSO) at concentrations of 0.005-5,000 µg/plate without mammalian metabolic activation (-S9) and 0.005-50 µg/plate with mammalian metabolic activation (±S9). Strains TA98, TA100, TA1535, TA1537, and TA2538 were evaluated for mutagenicity at 0.05-5.0 µg/plate (+S9) and all except TA100 at 0.00167-0.167 µg/plate (-S9). **There were no reproducible, dose-related differences in the number of revertant colonies in any tester strain at any dose level/condition compared to the vehicle controls.**

In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 44389704), L5178Y TK +/- mouse lymphoma cells cultured in vitro were exposed to triclosan (>99% a.i.) in dimethylsulfoxide (DMSO) at concentrations ranging from 1 to 25 µg/mL without metabolic activation (-S9) and from 1 to 20 µg/mL with mammalian metabolic activation (+S9). Treatment levels were selected based on a preliminary cytotoxicity test conducted at 1 to 250 µg/mL with and without activation. **Triclosan was negative for inducing forward mutations at the TK locus in mouse L5178Y cells both with and without metabolic activation.**

B. Chromosome Aberrations

In a mammalian cell cytogenetics, chromosome aberration assay (MRID 47276601), Chinese hamster ovary cells (CHO strain K_1-BH_4) were exposed to triclosan (>99% pure; Unilever sample number S15155 T01) and dissolved with DMSO. Concentrations of 0.1, 0.3, 0.5, and 1.0 µg/mL and 4.8, 9.5, 19.0, 30.0, and 38.0 µg/mL were tested for the cultures without and with metabolic activation from Aroclor 1254-induced rat livers for

24 and 6 hours, respectively. Cells were harvested 24 hours after treatment and analyzed for chromosomal aberrations. **There was no evidence of chromosome aberration induced over the background.**

In an *in vitro* cytogenetic assay (MRID 43740801), Chinese hamster lung fibroblasts were exposed to FAT 80'023/Q (triclosan: 99-100%) nonactivated doses of 1 µg/ml (7-hour cell harvest), 0.1-3 µg/ml (18-hour harvest), or 3 µg/ml (28-hour harvest) and S9-activated concentrations of 3 µg/ml (7- and 28-hour cell harvests) or 0.1-3 µg/ml (18-hour harvest). The S9 fraction was derived from Aroclor 1254 induced Wistar male rat livers and FAT 80'023/Q was delivered to the test system in ethanol. **Nonactivated triclosan at 1 and 3 µg/ml (18-hour harvest) induced a dose-related increase in the yield of cells with abnormal chromosome morphology. The response was significant ($p \leq 0.001$) at the higher concentration. A significant increase ($p \leq 0.001$) was also seen at 3 µg/ml (28-hour harvest). The most frequently observed type of chromosome damage was exchange figures. In the presence of S9 activation, nonsignificant but concentration dependent increases in cells bearing exchange figures were also seen at 1 and 3 µg/ml (18-hour harvest). Higher concentrations ($\geq$ 6 µg/ml –S9; $\geq$ 10 µg/ml +S9) were severely cytotoxic (< 1% survival). The data are, therefore, sufficient to conclude that triclosan is active in this test system over a narrow range of test material doses.**

In an *in vivo* bone marrow cytogenetic assay (MRID 43740802), groups of six male and six female Wistar rats received a single oral gavage administration of 4000 mg/kg FAT 80'023/Q (triclosan: 99-100%). The test material was delivered to the animals as suspensions prepared in 1% carboxymethyl-cellulose. Animals were sacrificed 6, 24, and 48 hours following compound administration and bone marrow cells from ten animals per group (5 males and 5 females) were harvested and examined for the incidence of structural chromosome aberrations. No overt toxicity or cytotoxicity for the bone marrow was seen up to a dose that exceeded the limit dose of 2000 mg/kg. **There was no indication of a clastogenic effect at any sacrifice time.**

C. Other Genotoxicity

In an *in vitro* DNA synthesis assay (MRID 47276602), rat hepatocytes were exposed to 39317 (batch/lot#: CC # 14663-09) dissolved in DMSO. Hepatocytes were isolated from the liver of two male Fischer 344 rats by the two-step *in-situ* perfusion. Concentrations of 0, 0.05, 0.1, 0.25, 1.0, 2.5, 5.0, 10, 25.0, 50.0, 100.0, or 250µg/mL were tested for 18-20 hours. Cells were autoradiographed, and unscheduled DNA synthesis was evidenced by a net increase in black silver grain counts using an Artek 880 automated colony counter with microscope and connected to an Apple II computer for data analysis. The difference between the cytoplasmic grain count and the corrected grain count was calculated and the net nuclear grains (NNG) and the percentage of hepatocytes in repair were calculated. Cytotoxicity was observed at 2.5 µg/mL based on the preliminary toxicity test and precipitation was seen at ≥50 mg/mL. Hence 25 mg/mL was selected as the highest dose concentration for the UDS assay. **There was no evidence of induction of unscheduled DNA synthesis in rat primary hepatocytes over the background.**

D. Overall Conclusions

Triclosan was not mutagenic in bacteria (*Salmonella typhimurium*) or cultured mammalian cells (mouse lymphoma L5178Y TK+/-). Chromosome aberrations conducted *in vitro* showed conflicting results [i.e., negative in Chinese hamster Ovary cells but significant and biologically relevant increases in structural chromosome aberrations in Chinese hamster lung fibroblasts (V79) over a narrow concentration range near levels causing severe cytotoxicity]. However, this activity was not seen when the test material was evaluated in an *in vivo* bone marrow cytogenetic assay in mice up to a level that exceeded the limit dose. Similarly, triclosan did not induce unscheduled DNA synthesis in primary hepatocytes *in vitro* In addition, a series of at least four additional *in vivo* assays are on file with the registrant but not submitted to the agency, although the acceptability of these studies may be in question because many were performed before GLPs were in place and others may not satisfy FIFRA guidelines. Nevertheless, all were negative, including a dominant lethal assay in mice and multiple dosing cytogenetic assays in mice or Chinese hamsters. We, therefore, conclude that Triclosan has intrinsic mutagenic activity *in vitro* but this is not expressed in whole animals. Accordingly, there is no concern for mutagenicity at this time.

3. Structure Activity Relationship (SAR)

Triclosan is a member of the diphenyl ether class of chemicals. However, no appropriate analogs were available for comparison.

4. Subchronic and Chronic Toxicity

A. Subchronic Toxicity

a) In a 90-day feeding study in rats (MRID 00133545), groups of Sprague-Dawley rats (25/sex/dose) received Irgasan (triclosan) at dietary concentrations 0, 1000, 3000, and 6000 ppm. The 6000 ppm animals showed signs of liver damage as characterized by "fatty metamorphosis and cytomegaly." Similar to a lesser degree of liver effects were also seen in the 3000 ppm groups. **The low dose, 50 mg/kg/day, was a NOAEL.**

b) In a subchronic feeding study (MRID 43022605), CD-1 mice were fed triclosan (99.7% a.i.) daily at dietary levels of 0, 25, 75, 200, 350, 750, or 900 mg/kg/day for 13 weeks (main groups, 15 mice per group) or 0, 25, 350, or 900 mg/kg/day for 7 weeks (satellite groups, 20 mice in the control group and 10 mice per treatment group). Satellite groups were run concurrently with the main groups and were mainly used to provide clinical pathology data. Animals from the satellite groups were sacrificed after- 7 weeks of exposure.

Systemic toxicity was observed at all dose levels in a dose-related manner as evidenced by clinical pathology, organ weight changes, and increased incidence or severity of histopathological lesions (especially of the liver). Clinical pathology included significantly decreased erythrocytes, hemoglobin, and hematocrit at $\geq$ 25 mg/kg/day in males (68%—92% of controls) and at $\geq$ 75 mg/kg/day in females (73%-91%). Enzyme

changes, indicative of liver injury, included increased alkaline phosphatase (at ≥ 25 mg/kg/day; 1.5-4.4 fold increases in both sexes), alanine aminotransferase (at ≥ 200 mg/kg/day; 1.3-6.2 fold increases in both sexes), and aspartate aminotransferase (at ≥ 200 mg/kg/day; 1.5-2.4 fold increase in males). Absolute and relative liver/gallbladder weights increased 1.3-3.0 fold at ≥75 mg/kg/day in both sexes. Increased incidence or severity of histopathological lesions in the liver included hypertrophic hepatocytes, vacuolization, inflammation, necrosis, pigmented Kupffer cells and/or macrophages, mineralization, and chronic bile duct inflammation. These lesions were evident in males at ≥ 25 mg/kg/day and in females at ≥ 200 mg/kg/day. The severity of extramedullary hematopoiesis in the spleen increased in males (≥ 200 mg/kg/day) and in females (≥ 750 mg/kg/day).

Additional findings at higher dose levels included organ weight changes (kidney, adrenal gland, uterus, ovary, and salivary gland); clinical signs (hunched posture, thin appearance, and hypoactivity, pale appearance, and cold to touch); changes in body weight gain (a decrease to 60% and 83% in males and females, respectively, for weeks 1-6 in the satellite groups and to 83% and 67% in males and females, respectively, for weeks 1-13 in the main groups); and increased incidence or severity of cystic stomach hyperplasia, subacute kidney inflammation, uterine hypoplasia, hypertrophic adrenal cortex (males); uterine hypoplasia; chronic inflammation of the kidney (females); tubule *regeneration* of the kidney, mammary gland dilatation and epithelial hypoplasia (females), chronic heart inflammation (females); pigmented macrophages in the mandibular lymph node (males); hypercellularity of the marrow of the femur (males); and lymphoid hyperplasia in the cecum (females).

Based on changes in clinical chemistry and hematology parameters as well as lesions in the liver at the lowest dose level, the systemic toxicity LOAEL was 25 mg/kg/day; a NOAEL could not be determined.

c) In a 28-day oral toxicity study (MRID 44389707), a total of 40 mice (MAGf [SPF], 5/sex/dose) received technical triclosan admixed into pelleted feed at dose levels of 0, 50, and 1000 ppm (6.48 and 135.59 mg/kg/day in males, 8.25 and 168.78 mg/kg/day in females) for 4 weeks. Five males and 5 females were given the high dose and allowed to "recover" (feeding of non-treated feed) for 2 weeks. There were no reported effects on mortality, body weight, or food consumption. Hematological effects were observed in the high dose (135.59 mg/kg/day in males; 168.78 mg/kg/day in females), and included significant decreases in erythrocytes, hemoglobin, and hematocrit in males, and a significant decrease in hemoglobin in females. Both sexes showed significant increases in thrombocytes at this dose, the effects of which were not fully reversible after two weeks recovery. Clinical chemistry alterations (significant increases in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase; significant decrease in globulin fraction) were observed at the high dose in male and female mice. Elevated serum enzyme activities were evident after the two week recovery period. Absolute weight of the liver and liver/body weight ratio were significantly increased at the high dose in male and female mice. Histopathological examination of the liver showed an increased incidence of liver cell necrosis (as single cells or small cell groups), hemosiderosis of Kupffer cells in the vicinity, cytoplasmic vacuoles in hepatocytes, and

liver cell hypertrophy. The presence of necrosis was still evident (2/5 males and 3/5 females) after the recovery period.

Based on the biochemical and morphological effects of Irgasan treatment on the liver of male and female mice, a systemic LOAEL of 135.59 mg/kg/day for males and 168.78 mg/kg/day for females is assigned. The systemic NOAEL is considered to be 6.48 mg/kg/day in males, and 8.25 mg/kg/day in females.

B. Chronic Toxicity

a) In a chronic toxicity/oncogenicity feeding study (MRID 42027906) conducted in male and female Sprague-Dawley rats, [FAT 80'023 (triclosan, 99.0 % a.i.)] was administered in the diet at doses of 0, 300, 1000, or 3000 ppm (0, 15.3, 52.4, and 168.0 mg/kg/day in males ; 0, 20.0, 66.9, and 217.4 mg/kg/day in females) for 104 weeks. An additional group of 20 male and 20 female rats received triclosan in the diet at 6000 ppm (415.0 mg/kg/day [males] and 519.3 mg/kg/day [females]) for 52 weeks. No treatment related effects on mortality, clinical toxicity, ophthalmology, urinalysis, gross pathology, or neoplastic pathology were observed at any dose level tested. Erythrocyte count, hemoglobin concentration, and hematocrit were decreased in males at the 15.3, 52.4, and 168.0 mg/kg/day dose levels, and erythrocyte count was decreased in females at 66.9 and 217.4 mg/kg/day. Serum alanine and aspartate aminotransferase activities were increased in males at 168.0 mg/kg/day, and blood urea nitrogen was increased in females at 217.4 mg/kg/day. Hepatocellular hypertrophy was observed in males at all dose levels. Increased incidence of liver necrosis was observed in males at the 300, 1000, and 3000 ppm dose levels (5/85, 4/85, and 4/85 respectively compared to 1/95 in concurrent controls). The predominant residue of triclosan observed in blood and kidney was the sulfate conjugate of triclosan, while unconjugated triclosan was predominant in the liver. Residual levels of triclosan were proportional to the dose administered. **The systemic LOAEL was determined to be 3000 ppm (168.0 mg/kg/day) based on significant decreases in body weight in male and female rats and non-neoplastic changes of the liver (cytoplasmic inclusions and hepatocellular hypertrophy) in males at 3000 ppm (168.0 mg/kg/day). The systemic NOAEL was determined to be 1000 ppm (52.4 mg/kg/day).**

b) In an 18-month carcinogenicity bioassay in mice, 5 groups of male and female CD-1 mice (70 mice/sex/dose) received triclosan in the diet at dose levels of 0, 10, 30, 100, or 200 mg/kg/day. Fifty mice/sex/dose received dietary triclosan for 18 months, while the remaining 20 mice/sex/dose received dietary triclosan for only 6 months, after which time these mice were sacrificed. Blood samples were obtained from 10 mice/sex/dose from both the 6 month and 18 month dose groups at sacrifice, for determination of triclosan plasma levels. There were no significant signs of clinical toxicity at any dose level, and no significant effects of treatment on group mean body weight, food consumption, ophthalmology, or urinalysis. A dose-related increase in activity of alanine aminotransferase and alkaline phosphatase was observed in male and female mice at 100 mg/kg/day triclosan and above in both the 6 month and 18 month dose groups. Significant decreases in both albumin and total protein were observed in males at 6

months and in females at 18 months at doses of 100 mg/kg/day and above. Serum cholesterol was markedly reduced at all dose levels including the 10 mg/kg/day dose. Treatment-related hematological effects included increased reticulocyte count and platelet count in males and females at the 200 mg/kg/day dose. Mean liver weight (absolute and relative) was increased in both male and female mice at 30 mg/kg/day and above at both 6 and 18 months. An increased incidence of nodules and discoloration of the liver was observed in both male and female mice at 100 mg/kg/day and above. A dose-related increase in severity of hepatocellular hypertrophy was observed in both male and female mice at 30 mg/kg/day and above. Dose-related increases in incidence or severity of hepatocellular vacuolation/vesiculation and hepatic inflammation, necrosis, and microgranulomas was also observed.

5. Mode of Action Analysis

Introduction

The purpose of this mode of action section is to present and evaluate the data submitted by Ciba Specialty Chemicals in support of the postulated mode of action for triclosan-induced hepatocarcinogenesis observed in the mouse carcinogenicity study. These data will be evaluated using the Agency Guidelines for Carcinogen Risk Assessment (USEPA 2005), as well as the work of Klaunig et al. (2003). Table 2 lists the key events necessary, according to current scientific understanding, to establish that a chemical causes hepatocarcinogenesis by way of a mode of action involving peroxisome proliferator activated receptor alpha (PPARα).

Table 2. Key events for the mode of action of liver carcinogenesis in rodents induced by PPARά ligands (Permission requested from the publisher)[1].

Event	Relationship[a]	Weight of evidence	Specificity	Comments
1. Activation of PPARα	Causal	Strong	High	Extensive evidence for PPARα activation Extensive evidence that PPARα null mice are resistant to key events Limited evidence that null mice are resistant to tumors
2a. Expression of peroxisomal genes	Associative	Strong	High	A good biomarker for PP exposure Causal link to cell proliferation and tumors uncertain
2b. PPARα-mediated expression of cell cycle, growth and apoptosis	Associative	Weak	Low	Putative target gene identities are unknown
2c. Nonperoxisome lipid gene expression	Associative	Strong	Low	Mediates hypolipidemia
3a. Peroxisome proliferation	Associative	Strong	High	Causal link to cell proliferation and tumors uncertain Excellent biomarker
3b. Perturbation of cell proliferation (i) and apoptosis (ii)	Causal	Strong	Low	Provides an MOA for carcinogenesis Common to other nongenotoxic carcinogens
4. Inhibition of GJIC	Associative	Strong	Low	Not clear if hepatocyte proliferation regulates GJIC or vice versa GJIC inhibition seen with other nongenotoxic liver carcinogens
5. Hepatocyte oxidative stress	Associative	Weak	Low	Occurs under many circumstances May regulate hepatocyte growth or cause low levels of DNA damage
6. Kupffer cell-mediated events	Associative	Strong	Low	Occurs under many circumstances Independent of PPARα
7. Selective clonal expansion	Causal	Strong	Low	Promotion of spontaneous DNA damage is key event in MOA Seen with all carcinogens

[a]Relationship to hepatic tumors or key events leading to tumors. *Causal*: Required step for PPARα MOA, based on empirical evidence. *Associative*: Events that are occurring but may or may not be causally linked to the MOA. *Weight of evidence (strong, weak): Strong* is normally defined by having several studies which support that MOA, preferably with multiple PPARα agonists from multiple laboratories and with limited evidence of contradiction. *Weak* is normally defined by having a single study with a single PPARα agonist from a single laboratory or a significant amount of contradiction in the literature. *Specificity to PPARα-induced rodent hepatic tumors (high, low): High* is defined as unique to this PPARα MOA. *Low* is defined as not unique to PPARα MOA.

[1] Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, DeLuca JG, Lai DY, McKee RH, Peters JM, Roberts RA, Fenner-Crisp PA. (2003). PPARα agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol 33(6):655-780.

Key events

The key events for demonstrating a PPARα mode of action include activation of PPARα, increased cell proliferation (as measured by DNA synthesis), decreased apoptosis, clonal expansion, and the appearance of liver tumors. Other associative events include peroxisome proliferation defined as an increase in the volume density of peroxisomes and an increase in peroxisomal enzyme activity (IARC 1995; Klaunig et al. 2003), and increased expression of peroxisomal genes (e.g., increased acyl CoA oxidase activity (i.e., peroxisomal genes of β-oxidation). Additional data that are desirable to support the weight-of-evidence analysis for PPARα MOA include: hepatic CYP4A1 induction, increased palmitoyl CoA oxidase activity, hepatocyte hypertrophy and increased liver weights, increased microsomal fatty acid oxidation, increased hydrogen peroxide formation, and decrease in triglyceride levels.

Evidence in support of the key events listed in Table 2 above is summarized for each event.

1. *Activation of PPARα*

There is no direct evidence for the activation of PPARα provided. The lack of this data is not considered a critical gap in the understanding of the mode of action

2a. *PPARα –dependent regulation of genes encoding for peroxisomal enzymes*

Evidence in support of this event is from the study of Persohn et al. (1992) (MRID 45307507), where induction of CYP 3A and 4A was observed as well as increased regioselective testosterone hydroxylation and fatty acid β-oxidation from administration of triclosan to mice for 14 days. These data are summarized below from the report:

The Effect of Irgasan DP 300 on Selected Biochemical Parameters in **Male** Mouse Liver								
Test Group	Prot.	P-450	GST	FAO	11-OH	12-OH	EROD	PROD
1M	22.0± 4.7	11.4± 4.3	332± 52	963± 103	25.0± 6.6	62.4± 17.8	1.49± 0.63	0.76± 0.43
3M	27.6± 2.1*	17.6± 6.9	403± 101	974± 103	36.9± 7.3#	87.6 ±19.5	2.72± 0.92#	3.28± 1.66†
4M	27.6± 1.8*	19.2± 6.6*	311± 75	1568± 432†	56.7± 16.5†	186.5± 90.7†	3.52± 0.76†	4.57± 1.13†
5M	31.0± 3.4†	38.1± 4.5†	527± 80†	2381± 386†	92.5± 12.8†	391.3± 66.2†	5.63± 1.19†	10.50± 1.94†
6M	34.7± 3.5†	49.9± 10.9†	867± 111†	3269± 292	110.4 ±19.9 †	519.5± 69.7†	7.48± 1.97†	18.15± 5.66†

Data from pages 29-32 of the report.

Prot. = microsomal protein (mg/g liver); P-450 = cytochrome P-450 (nmol/g liver); GST = glutathione-S-transferase (nmol/min/g liver); FAO = fatty acid ß-oxidation (nmol/min/g liver); 11-OH and 12-OH = lauric acid hydroxylation (nmol/min/g liver); EROD = ethoxyresorufin O-de-ethylase (nmol/min/g liver); PROD = pentoxyresorufin O-depentylase (nmol/min/g liver).
* p<0.05; # p<0.01; † p<0.001 vs. control by Dunnett's test.
Test groups: 1M = control; 3M = 18.4 mg/kg/day; 4M = 53.2 mg/kg/day; 5M = 249.3 mg/kg/day; 6M = 793.8 mg/kg/day.

As these data show, significant increases were observed in activities of all biochemical parameters listed in male mice, including lauric acid hydroxylation (11-OH, 12-OH) and EROD/PROD activities at the lowest dose (≥18.4 mg/kg/day), fatty acid β-oxidation (≥53.2 mg/kg/day). In female mice, increases in these parameters were observed to be increased significantly at the mid dose (271.3 mg/kg/day for female mice) and above.

Results of testosterone hydroxylation measurements are shown below:

Metabo-lites	Effect of Irgasan DP 300 Treatment on P-450 Dependent Testosterone Hydroxylation					
nmol/g /min	0 mg/kg /day	18.4 mg/kg /day	53.2 mg/kg /day	249.3 mg/kg /day	793.8 mg/kg /day	High dose recovery
2ß-OH	2.5±0.5	4.9±2.9*	5.7±2.9*	20.6± 5.2***	29.1± 3.5***	1.5± 0.2***
6ß-OH-T	30.0± 8.3	61.3± 40.8*	57.4± 26.8*	259.5± 59.1***	327.8± 31.7***	13.0± 1.8***
15ß-OH-T	3.1±0.8	4.4±2.0	5.5±2.7*	16.6 ±4.4***	22.7± 2.5***	1.6±0.9*
6 -OH-T	4.6±2.2	4.3±1.2	3.0±0.6	5.0±2.0	6.0±3.0	1.9±0.2*
7 -OH-T	10.6±4.8	14.7±6.9	15.6±6.8	20.7 ±7.5*	36.4± 8.7***	6.9±2.4
16 -OH-T	8.8±2.7	12.7±6.9	15.5 ±2.6*	19.1 ±3.7***	19.3 ±5.6**	3.7±1.2
androstene-dione	17.3 ±4.0	27.0 ±7.2	17.1 ±4.7	28.6 ±6.7	31.1 ±9.6	10.9 ±2.1
16ß-OH-T	2.2±1.8	3.5±4.2	3.9±1.8	9.5±5.3	16.8 ±2.5*	0.6±0.5
Total Activity	79.0 ±15.8	132.8 ±64.9*	123.7 ±41.2*	379.4 ±66.1***	489.1 ±51.0***	40.0 ±5.3***

As shown, total microsomal hydroxylation of testosterone was significantly increased at all dose levels tested in male mice, and the increases were dose-dependent. Formation of the 2ß-, 6ß-, 15ß-, and 16ß- metabolites were increased 11.6-fold, 10.9-fold, 7.3-fold, and 7.6-fold, respectively, at the high dose level. The prominent induction of formation of these metabolites is similar to that observed after treatment with the model inducers phenobarbital and pregnenolone 16-carbonitrile. Hydroxylation of testosterone at the 7 position (alpha configuration) is associated with CYP2A1 and isoenzymes of the peroxisome proliferator

inducible P-450 gene family CYP4A in the rat. The study results showing a 3.4-fold induction of 7-alpha-hydroxylation by dietary administration of Irgasan DP 300 in male mice supports the conclusion of a peroxisome-proliferator effect of the chemical. Previously mentioned induction of fatty-acid hydroxylation by Irgasan also supports this conclusion.

The table below shows induction of cytochrome P-450 3A and 4A in response to oral administration of Triclosan, also measured in the study of Persohn et al. (1992) (MRID 45307507). Induction of both CYP3A and CYP4A is noted after oral administration of Triclosan for 14 days.

Microsomal Fraction, female	CYP Detected by antibody d15 (1A1 and 1A2)	CYP Detected by antibody p6 (3A1 and 3A2)	CYP Detected by antibody clo4 (4A)
0 mg/kg/day	100	100	100
19.8 mg/kg/day	70	540	241
271.3 mg/kg/day	48	3293	703
1105.6 mg/kg/day	70	5851	852

2b. *PPARα-dependent expression of cell cycle growth and apoptosis*

Evidence for this event is not required to support the proposed mode of action.

2c. *PPARα-dependent expression of nonperoxisomal fatty acid metabolism genes*

Data in support of this event are from Persohn et al. (1992) (MRID 45307507), in which dose-dependent and significant increases in cyanide-insensitive peroxisomal fatty acid β-oxidation were observed following oral treatment with Triclosan to mice. As noted above in item 2a, dose-dependent increases of 162, 247, and 339% were observed in the activity of this enzyme in male mouse liver at the 53, 2[illegible], and 793 mg/kg/day dose levels. Activity of cyanide-insensitive peroxisomal fatty acid β-oxidation was also increased in female mouse liver at the top two dose levels by 416 and 349% respectively. Following a four-week recovery period, the activity of this enzyme returned to normal. The significant increase in the activity of this enzyme supports the mode of action of Triclosan as a peroxisome proliferator.

3a,b. *Peroxisome Proliferation, perturbation of cell proliferation and/or apoptosis*

Data in support of this key event are from a study conducted by Elridge (1995) entitled "Cell proliferation in Rodent Liver" (MRID 45307508). The purpose of this study was to examine whether cell proliferation was induced in mice which had been used in a subchronic toxicity study. Formalin-fixed tissue was obtained from the 45- and 90-day time points of the subchronic toxicity study for analysis of cell proliferation using PCNA staining. As the data below show, an increase in labeling index was apparent at the 200 mg/kg/day dose and above at both the 45 and 90 day time points for male and female mice.

Cell Proliferation in Male and Female Mice Administered Dietary Irgasan for 90 Days							
Group (males, mg/kg /day)	Mean Labeling Index	SEM	Fold Increase Over Controls	Group (females, mg/kg /day)	Mean Labeling Index	SEM	Fold Increase Over Controls
0	0.035	0.016	-	0	0.042	0.036	-
25	0.008	0.008	0.0	25	0.046	0.015	1.1
75	0.167	0.082	4.8	75	0.065	0.009	1.5
200	0.124	0.031	3.5	2 0 0^^^Document Error^^^ Error^^^	0.140	0.050	3.3
350	0.398	0.063	11.0	350	0.256	0.218	6.1
900	0.536	0.195	15.0	900	0.300	0.060	7.1
Cell Proliferation in Male and Female Mice Administered Irgasan in the Diet for 45 Days							
Group (males, mg/kg /day)	Mean Labeling Index	SEM	Fold Increase Over Controls	Group (females, mg/kg /day)	Mean Labeling Index	SEM	Fold Increase Over Controls
0	0.090	0.018	-	0	0.058	0.029	-
25	0.112	0.089	1.2	25	0.064	0.021	1.1
350	0.292	0.096	3.2	350	0.242	0.072	4.2
900	0.726	0.218	8.0	900	0.380	0.080	6.6

[a]data obtained from Table III of the report (no page number). N = 5 except for controla at 90 days, where N = 7 for males and N = 5 for females.

5,6. *Hepatocyte oxidative stress/Kupffer cell mediated events*

Responses such as oxidative stress and Kupffer cell mediated events are considered secondary responses to cell injury and are not specific responses to chemicals operating through a peroxisome proliferating mode of action. However, evidence is in the available data for Triclosan from a subchronic oral toxicity study in the mouse (Trutter, 1993 [MRID 43022605]). Histopathological examination of liver tissue showed increased incidence of Kupffer cell pigment at doses of 75 mg/kg/day and above in male mice and at 200 mg/kg/day and above in female mice.

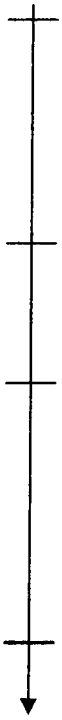
7. *Selective Clonal Expansion*

Hepatocellular tumors only were reported in the 18-month mouse carcinogenicity study, and there were no reports of an increase in basophilic foci in the review. If this information were available, it would strengthen the proposed mode of action for Triclosan.

Temporal association

The sequence for the proposed key events in triclosan-induced liver tumor formation in mice is outlined in Figure 1. Increased liver weights and liver hypertrophy were observed in studies of 2 and 4 weeks duration following oral administration of triclosan to mice (MRIDs 45307507, 44389707). This temporal precedence strengthens the proposed mode of action because tumors were observed after 78 weeks of administration of triclosan to mice. Increased peroxisome proliferation was observed after 2 weeks of triclosan administration (MRID 45307507). Signs of hepatotoxicity were also observed at 2, 4, and 13 weeks after triclosan administration and were observed until tumor formation (week 78).

Oral Exposure of mice to Triclosan



2 Weeks	Increased absolute/relative liver weight, proliferation of smooth endoplasmic reticulum, peroxisome proliferation, increased regioselective testosterone hydroxylation, increased CYP3A1 and 3A2, and 4A (250 mg/kg/day and above) (MRID 45307507)
4 Weeks	Increased absolute/relative liver weight, liver hypertrophy, increased ALT, AST, increased cell necrosis 135-168 mg/kg/day) (MRID 44389707)
13 Weeks	Increased absolute/relative liver weight, hepatocellular hypertrophy, increased ALT, AST, (200 mg/kg/day and above) liver cell necrosis (75 mg/kg/day and above), pigmented Kupffer cells (25 mg/kg/day and above; Trutter, 1993). Increased cell proliferation (75 mg/kg/day and above) (MRID 45307508)
78 Weeks	Increased absolute liver weight, liver hypertrophy, liver tumors; (30 mg/kg/day and above [M]; 100 mg/kg/day and above [F])

Dose-Response Concordance

In the mode of action analysis, establishing whether the dose-response relationship for any key step in the postulated mode of action parallels that of other key steps is essential. This is known as establishing dose-response concordance. Ideally, key events should occur in a dose-dependent manner such that "the key [precursor] events forecast the appearance of tumors at a later time or higher dose" (USEPA 2005). The significantly increased incidence of adenomas, carcinomas, and/or adenomas/carcinomas combined at 30 mg/kg/day in males is consistent with the proposed mode of action because liver tumors are not seen at lower doses than precursor events in shorter-term studies.

In general, the measured precursor events occur at the same doses as the tumor response. This fact strengthens the dose-response concordance for the 18-month feeding study in CD-1 mice. Table 3 summarizes the available information on the key events measured in the 18-month carcinogenicity study in mice. Data were only available from the FDA review document and from the December 2000 Triclosan Expert Panel Report, due to legal constraints.

Table 3. Key events measured in the 18-month bioassay in CD-1 mice (See, 1996; Triclosan Expert Panel, 2000)

Dose (mg/kg BW/day; M/F)	Absolute Liver Weights		Hepatocellular Necrosis (% incidence)[1]		Hepatocellular Hypertrophy (% incidence)[1]		Liver Brown Pigment (% incidence)		Neoplasms (See, 1996) Incidence (%)					
									Adenoma		Carcinoma		Combined	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0	1.97±0.52	1.52±0.27	12	10	0	0	0	0	5 (10)	0 (0)	2 (4)	0 (0)	6 (12)	0 (0)
10.1/9.9	2.22±0.84	1.61±0.29	10	8	20	3	0	0	7 (14)	1 (2)	3 (6)	0 (0)	10 (20)	1 (2)
30.2/30.1	2.38±0.68*	1.85±0.72*	13	9	45	25	7	5	13 (26)*	3 (6)	6 (12)	1 (2)	17 (34)*	3 (6)
100.4/100.1	3.73±0.95*	2.72±0.97*	39	25	75	43	73	48	22 (44)***	6 (12)**	11 (22)**	1 (2)	32(64)***	6(12)*
201.4/201.3	5.21±1.28*	3.76±1.06*	45	35	57	65	80	80)	26 (53)***	11 (25)**	24 (49)***	14 (28)***	42 (86)***	20 (40)***

* stated to be statistically significant from FDA review; p values not provided.

***$p < 0.001$ from FDA review. Data reproduced from page 8 of the review.

[1]percent incidence was estimated from graphical data provided in the Triclosan Expert Panel Report; no tabular data were available. However, the Expert Panel's report is generally consistent with the FDA's review of these data.

Other Modes of Action

It has been suggested that Triclosan tumorigenesis may also be associated with induction of xenobiotic metabolizing enzymes or a sustained regenerative cellular proliferative response. A mutagenic mode of action can be ruled out based on the overall negative *in vivo* genotoxicity database for Triclosan. A cytotoxic mode of action is ruled out based on the lack of evidence supporting a sustained regenerative cellular proliferative response. Cell proliferation data from the 45- and 90-day time points are more consistent with a mitogenic response and not with a cytotoxic mode of action, because cellular proliferation decreased at 90 days relative to 45 days. An increased and sustained cellular proliferative response would be expected if a cytotoxic mode of action was operative.

Conclusions

Based on the data presented here and the current state of the science with regard to peroxisome proliferators, it is reasonable to conclude that triclosan-induced hepatocarcinogenesis in CD-1 mice is mediated through activation of PPARα. Two of the three known causal events in the proposed mode of action, i.e., increased cell proliferation and tumor formation, have been shown to occur in short- and long-term studies, respectively. In addition, several key associative events, namely increased expression of enzymes associated with PPARα activation have been demonstrated in several short-term studies of triclosan.

Human Relevance

The overall weight of the evidence supports activation of PPARα as the mode of action of hepatocarcinogenesis for triclosan in mice. Liver tumors were not observed in rats or hamsters after administration of similar or higher concentrations of triclosan in the diet. The animal database also indicates that some key events of the mode of action are observed in rats but at much higher dose levels than mice. Because human cells contain PPARα and human hypolipidemic drugs work through this receptor, the mode of action of triclosan-induced hepatocarcinogenesis is qualitatively plausible in humans.

The proposed mode of action in adults, however, is not quantitatively plausible when several factors are considered. First, while PPARα is found in human hepatocytes, its expression and activity is approximately 10-fold lower than that of mouse hepatocytes (Palmer et al. 1998). Second, peroxisome proliferators (including hypolipidemic drugs) that are carcinogenic in rodents have not been shown to be carcinogenic in other animal species, including humans (Lai 2004). Third, in addition to these species differences, current research suggests that human liver is less susceptible to peroxisome proliferation, probably due to the low level of PPARα activity in humans (Cattley 2004). This has been shown in cultured human hepatocytes as well (IARC 1995). While the proposed mode of action for liver tumors in mice is theoretically plausible in humans, hepatocarcinogenesis by this mode of action is quantitatively implausible and unlikely to take place in humans when quantitative species differences in PPARα activation and toxicokinetic and toxicodynamic factors are taken into account (Klaunig et al. 2003).

V. WEIGHT-OF-THE-EVIDENCE CONSIDERATIONS

1. Carcinogenicity

RAT

•No treatment-related increase in tumors was seen in male or female Sprague-Dawley rats at doses up to 3000 ppm.

•Adequacy of Dosing: The dose of 3000 ppm (168/217.4 mg/kg/day, M/F) in the rat chronic toxicity/carcinogenicity study was considered to be adequate and not excessive for purposes of carcinogenicity testing. Mortality was not adversely affected in this study. Mean body weight of male rats was significantly decreased only up to week six of the study (~6%) and mean body weight of female rats was decreased between 5-10% from weeks 3-76 of the study. Significantly increased incidence of non-neoplastic lesions of the liver in males (cytoplasmic inclusions and hepatocellular hypertrophy) were also observed at 3000 ppm as was an increased incidence of renal calculi at this dose.

HAMSTER

•No treatment-related increase in tumors was seen in male or female BioF1D Alexander Syrian hamsters at doses up to 250 mg/kg/day.

•Adequacy of Dosing: Dosing at the high dose of 250 mg/kg/day was considered adequate and not excessive. This was based on increased mortality in high dose males only after week 80, decreased body weight (84-85% and 89-90% of controls, males/females, respectively) and body weight gain in both sexes (46-53% of controls through week 90), hematological alterations (increased urea nitrogen, increased serum triglycerides), and non-neoplastic lesions of the kidney (distended medullary tubules, increased incidence and severity of nephropathy), increased incidence of stomach lesions, and partial depletion of one or more generation of germ cells in the testis.

MOUSE

• In male CD-1 mice, the incidence of liver adenomas, carcinomas, and combined adenomas and/or carcinomas for the control, 10, 30, 100, and 200 mg/kg/day dose groups were as follows:

Adenomas:	5/50 (10%), 7/50 (14%), 13/50 (26%), 22/50 (44%), 26/49 (53%)
Carcinomas:	2/50 (4%), 3/50 (6%), 6/50 (12%), 11/50 (22%), 24/49 (49%)
Combined:	6/50 (12%), 10/50 (20%), 17/50 (34%), 32/50 (64%), 42/49 (86%)

Male rats had significant increasing trends at $p<0.005$ for adenomas, carcinomas, and combined adenomas and/or carcinomas. There were significant differences in the pair-wise comparisons of the 30, 100, and 200 mg/kg/day dose groups with the controls, for combined liver adenomas and/or carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 100 and 200 mg/kg/day dose groups with the controls, for liver adenomas and carcinomas, all at $p<0.01$. The incidence of adenomas and adenomas and/or carcinomas

combined exceeded historical control incidences (10% for adenomas; 17% for combined) at the ≥10 mg/kg/day dose level, but were statistically significant at the ≥30 mg/kg/day dose level. The incidence of carcinomas exceeded the historical control incidence (7%) at ≥30 mg/kg/day, but was statistically significant at ≥100 mg/kg/day. The CARC considered the liver tumors in male rats to be treatment-related.

• In female CD-1 mice, the incidence of liver adenomas, carcinomas, and combined adenomas and/or carcinomas for the control, 10, 30, 100, and 200 mg/kg/day dose groups were as follows:

Adenomas:	0/50 (0%), 1/50 (2%), 3/50 (6%), 6/50 (12%), 11/50 (22%)
Carcinomas:	0/50 (0%), 0/50 (0%), 1/50 (2%), 1/50 (2%), 14/50 (28%)
Combined:	0/50 (0%), 1/50 (2%), 3/50 (6%), 6/50 (12%), 20/50 (40%)

Female rats had significant increasing trends at $p<0.005$ for adenomas, carcinomas, and combined adenomas and/or carcinomas. There were significant differences in the pair-wise comparisons of the 100 and 200 mg/kg/day dose groups with the controls, for liver adenomas and combined adenomas and/or carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 200 mg/kg/day dose groups with the controls, for liver carcinomas, at $p<0.01$. The incidence of adenomas and adenomas/carcinomas combined exceeded historical control incidences (1% for adenomas; 1% for combined) at the ≥10 mg/kg/day dose level, but were statistically significant at the ≥100 mg/kg/day dose level. The incidence of carcinomas exceeded the historical control incidence (0%) at ≥30 mg/kg/day, but was statistically significant at 200 mg/kg/day. The CARC considered the liver tumors in female rats to be treatment-related.

• Adequacy of Dosing: The dose level of 200 mg/kg/day was considered adequate in both sexes and not excessive for carcinogenicity testing. At ≥30 mg/kg/day there was a dose-related increase in liver weight and hepatocellular hypertrophy. In addition, increased mortality was observed in male mice at 100 mg/kg/day and in female mice at 200 mg/kg/day.

2. Mutagenicity

• Triclosan has intrinsic mutagenic activity *in vitro* but this is not expressed in whole animals. Accordingly, there is no mutagenicity concern for triclosan.

3. Structure Activity Relationship (SAR)

No appropriate analogs were available for comparison.

4. Mode of Action

The overall weight-of-the-evidence supports activation of peroxisome proliferator activated receptor alpha (PPARα) as the primary mode of action for triclosan-induced hepatocarcinogenesis in mice. Key precursor events and the tumor response in mice were concordant with respect to both time and dose. While the proposed mode of action for liver tumors in mice is theoretically plausible in humans based on a functional PPAR-alpha receptor, it is quantitatively implausible and unlikely to take place in humans when quantitative species

differences in PPARα activation and toxicokinetic and toxicodynamic factors are taken into account (i.e. the formation of liver tumors in mice has no relevance to humans) (Klaunig et al 2003). The data did not support either a mutagenic mode of action or a cytotoxic mode of action that is consistent with a sustained regenerative cellular proliferative response.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Final Guidelines for Carcinogen Risk Assessment (March 29, 2005), the CARC classified Triclosan as "Not Likely to be Carcinogenic to Humans". This decision is based on the weight of evidence that supports activation of peroxisome proliferator activated receptor alpha (PPARα) as the primary mode of action for triclosan-induced hepatocarcinogenesis in mice. The data did not support either a mutagenic mode of action or cytotoxic mode of action that is consistent with a sustained regenerative cellular proliferative response. While the proposed mode of action for liver tumors in mice is theoretically plausible in humans based on the availability of a functional PPAR-alpha receptor, hepatocarcinogenesis via this mode of action is quantitatively implausible and unlikely to take place in humans based on quantitative species differences in PPARα activation and differences in toxicokinetics.

VII. QUANTITATION OF CARCINOGENIC POTENTIAL

The quantification of risk is not required.

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