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

MAR 5 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg.#100-502; Irgasan DP 300 (Triclosan); Request for Removal of Label disclaimer "Do Not Use For Baby Diaper Laundry". CASWELL#186A

FROM: William Dykstra, Toxicologist
Toxicology Branch, HED (TS-769) WJD for LDC 2/27/81

TO: A.E. Castillo (32)
Registration Division (TS-767) WJB

Recommendations:

1) All submitted I.B.T. studies must be validated before initiation of their review. The following studies have been identified as I.B.T., but additional I.B.T. studies on Irgasan may exist.

   a) J-4915; 18-Month Dermal Carcin.
   b) A-8434; Subacute Dermal
   c) C-5416; Subacute Dermal
   d) 602-2220
   e) 621-4784; 90-Day Dermal Monkeys
   f) 622-4554; 90-Day Rats
   g) 622-5278; 18-Month Oral Mice
   h) 622-6047; 2-Year Oral Rats
   i) P-7113, J-7112; Three Phase Reproduction Study

2) The validated I.B.T. studies (or repeated studies) and the toxicology studies requested by Charles Frick in memo of 2/11/77 are required to be submitted before any conclusions can be made regarding the label disclaimer.

3) With respect to the Irgasan DP 300 skin absorption study in the rabbit, Table 12, showing the Irgasan DP 300 content of diapers utilized in the study, demonstrates that the diapers of male and female rabbits of Test A contained an average of 15 ppm and the diapers of male and female rabbits of Test B contained an average of 20 ppm. The diapers originally contained about 57 ppm. These data suggest that absorption or loss from the diaper is significant. Did test animals A absorb 2.1 mg from a total of 2.9 mg and test animals B absorb 1.85 mg out of a total of 2.9 mg? The registrant is requested to address the above concerns.

4) With respect to the 90-day bathing of newborn rhesus monkeys with 0.1% Triclosan soap solution, the area of the animals exposed to treatment (the trunk) is less than the area of exposure during washing for a human infant. The area and time of dermal exposure (5 minutes/day) may not be appropriate for extrapolation to washed human infants.

   In addition, since the Triclosan concentration (0.1%) used in the study is the same concentration used to bathe human infants, the margin of safety for human infants is not apparent.

5) The studies submitted are acceptable as core-minimum data or supplementary data.

6) The registrant is required to analyze technical Triclosan for impurities of chlorodibenzodioxins and chlorodibenzofurans.

Review:

A. Recently Submitted Studies on 6/18/80

1) Irgasan DP 300 (Triclosan) Skin Absorption in the Rabbit (Huntingdon Research Center; HC#1-289N; November 28, 1977)

   Test Material:

   The following materials were received from Ciba-Geigy Corporation, 444 Saw Mill River Road, Ardsley, New York.

   1. White cloth diapers washed with fabric softner containing 2.5% Triclosan at a ratio of 4 ounces of softner per 100 pounds of fabric.

   2. White cloth diapers washed with an identically formulated fabric softner containing no Triclosan at a ratio of 4 ounces of softner per 100 pounds of fabric.

   Methods:

   Thirty-six(36) (18 male, 18 female) healthy New Zealand White albino rabbits obtained from Skippack Laboratory Animals, Inc., Skippack, PA., were used as experimental animals.

   Rabbits were individually housed in suspended stainless steel wire meshed cages in a room controlled for temperature and light cycle (0600 through 1800 hours). Purina Rabbit Chow and water were available ad libitum. Urine and feces dropped on to a cheese cloth screen and excess urine was caught in stainless steel pans. Sample of feces were obtained from areas of the screen not containing urine.

   After a seven (7) day acclimation period to laboratory conditions, the back and flank area of each rabbit was clipped free of hair with Oster electric clippers equipped with a number 40 blade (Friday, November 4, 1977).

The rabbits were then randomly assigned to the following groups and received the indicated treatment regimen:

Group	Male	Female	Skin	Diaper
I	6	6	Abraded	Control
II	6	6	Abraded	Irgasan
III	6	6	Intact	Irgasan

Monday, November 6, 1977:

All rabbits were bled via venipuncture of the medial ear vein. Blood was collected in Vacutainer (B-D) tubes treated with heparin. Blood was immediately frozen.

Thursday, November 10, 1977:

Members of the Huntingdon Research Center Staff were given sterile containers and instructions for obtaining a "clear catch" urine specimen the following morning. The instructions were as follows:

1. Allow a small amount of urine to flow through urethra.
2. Catch a large a sample of urine as possible in container.
3. Finish voiding.
4. Refrigerate urine.

Rabbits were shaved again to remove hair which had grown in.

Friday, November 11, 1977:

Control and treated diapers were cut into eight(8) pairs of approximately the same size. The diapers were large enough to cover at least twenty(20) percent of the total body surface.

At 0900 hours the diapers were laid across the shaved area and soaked with fifteen(15) milliliters of human urine. The diaper was then covered with a cellophane wrap and a rubberized elastic bandage. The bandage was then held in place with adhesive tape. The above procedure was repeated at 1300 and 1700 hours. The 1700 dressing remained in place overnight.

Blood was collected in Vacutainer (B-D) tubes treated with heparin via venipuncture of the medial ear vein at each change. Blood was immediately frozen.

Saturday, November 12, 1977:

The bandage was removed from each animal and blood obtained and frozen. Urine and fecal specimens were collected from each animal and frozen.

One-half the animals from each group were sacrificed via air embolism and portions of the following tissues collected and frozen: liver, brain, kidney, heart, spleen, sartorius muscle, and fat.

During the study, composite samples of human urine were frozen.

All frozen samples were submitted to Ciba-Geigy on November 22, 1977.

2) Irgasan DP 300 Skin Absorption Study in the Rabbit. Final Analytical Report (Ciba-Geigy; 3/6/78)

Trace amounts of Irgasan DP 300 were detected in some of the blood samples and feces samples but in most cases none was present.

Small amounts of Irgasan DP 300 were detected in the urine collecting samples. The amount found in urine ranged from 0.1 to 3.8 ug. The amount of urine voided by the rabbits during treatment was not presented.

Since all the tissues from each individual animal were shipped in the same containers, cross contamination was possible.

Therefore, only the liver and kidney samples were analyzed. No Irgasan DP 300 was detected in the liver samples and only trace quantities were detected in the kidney samples.

Representative, urine saturated, diaper samples were also received from the testing laboratory and were analyzed for their Irgasan DP 300 content. The total amount of Irgasan DP 300 on the treated diaper samples ranged from 100-400 ug. Three of the control samples contained small amounts of Irgasan DP 300 (2-10 ug). The latter may have been due to contamination since no Irgasan DP 300 was detected in any of the other control samples.

Conclusion:

Table 12, showing the Irgasan DP 300 content of diapers utilized in the study, demonstrates that the diapers of male and female rabbits of Test A contained an average of 15 ppm and the diapers of male and female rabbits of Test B contained an average of 20 ppm.

The diapers originally contained about 57 ppm. These data suggest that absorption is signficant. Did test animals A absorb 2.1 mg from a total of 2.9 mg and test animals B absorb 1.85 mg out of a total of 2.9 mg?

Classification: Supplementary Data

(a) Registrant is required to address the question of absorption or the loss of Irgasan.

3) Urine extraction of Irgasan DP 300 from Diapers (Ciba-Geigy D&C Division; Project: 77 2 12 35; 1/17/78

Results show that average levels of Irgasan DP 300 extracted into urine off of diapers containing (2.9 mg) 57 ppm Irgasan DP 300 were 0.0667 mg (respectively 2.3% of the total amount of Irgasan DP 300 on the diapers) after 4 hours and 0.075 mg (2.64%) after 16 hours.

Conclusion:

Small amounts of Irgasan DP 300 were extracted from diapers by human urine.

Classification: Supplementary Data

4) Use of the Mouse Spot Test to Investigate the Mutagenic Potential of Triclosan (L.B. Russell and C.S. Montgomery; Biology Division, Oak Ridge National Laboratory; 3/21/80)

Triclosan was tested in the mouse in vivo somatic mutation test (spot test) by intraperitoneal injeciton on day 9 1/4 or 10 1/4 post-conception.

Results:

Although the dose range tested overlapped the toxic, the frequency of presumed somatic mutations was not significantly greater in the experimetal groups than in the methanol-injected controls; and the results rule out with 95% confidence a spot incidence five or more times greater than the control incidence.

Conclusion:

Ingrasan DP 300 produces readily detectable toxic effects in embryos of dams receiving a single i.p. injection of 8 mg/kg on day 9 1/4 or 10 1/4 post-conception and slight toxic effects even at a weighted-average exposure of 3.2 mg/kg; but there is no evidence of induced mutagenicity at 8 mg/kg. Because of survival problems, it is impractical to study mutagenicity at higher exposure levels.

Classification: Core-Minimum Data

5) Genetic Activity of Irgasan DP 300 in the MP-1 Strain of S. cerevisiae (R. Fahrig; June 22, 1978)

The ascertain whether the yeast cultures treated for 210 minutes with 0.2 mg. Irgasan DP 300/ml differ significantly from control culture, an evaluation of the data using the T-test was made. The results summarized in the following table show that Irgasan DP 300 has an effect in the mutation and intergenic recombination system, but not in the interallelic recombination system.

Genetic Alteration	Number of Exp.	Survival	Colonies of genetically altered cells per survivors	Level of significance (P)
Experiment				
mutation	3	82 ± 7	3.33 ± 0.16	< 0.01
intergenic rec.			0.14 ∓ 0.08	< 0.02
interallelic rec.			3.04 ∓ 0.44	> 0.2
Control				
mutation	3	100	1.86 ± 0.52	
intergenic rec.			0.20 ∓ 0.03	—
interallelic rec.			2.57 ∓ 0.14	—

Conclusion:

Irgasan DP 300 shows weak, but definite mutagenic and recombinogenic activity in the strain MP-1 of S. cerevisiae. The substance is active per se, i.e., it does not need metabolic activation.

Classification: Supplementary Data

(a) Only one dose tested and results are contradicted by #7.

6) The effect of Irgasan DP 300 in the "Mammalian Spot Test": an in vivo method for the detection of genetic alterations in somatic cells mice (R. Fahrig; June 22, 1978)

Results:

At a control frequency of about 0.1%, 50 mg/kg Irgasan DP 300 induced about 2.4% color spots of genetic relevance.

Conclusion:

The dose of Irgasan DP 300, 50 mg/kg, used to detect color spots was maternally lethal in the previous (#4) review of the same type study. Therefore, the results of this study are questionable.

Classification: Supplementary Data

7) Mutagenicity Test on Saccharomyces Cerevisiae MP-1 in vitro with FAT 80 023/A (Irgasan DP 300) (CIba-Geigy Ltd; GU 2.3; November 27, 1978; 78/3402)

FAT 80 023/A was tested for mutagenic effects on the multi-purpose MP-1 strain of S. cerevisiae. The concentrations of the substance used were 10, 20, 30, 40, 50, 60 and 200 mg/L. Positive control used was 4-nitroquinoline-N-oxide.

This test system permits the detection of mutagenic effects that induce intergenic recombination (mitotic cross-over), intragenic recombination (mitotic gene conversion) or forward mutations (point mutations) in yeast cells. The recombinants resulting from mitotic cross-over are recognizable by their adenine deficiency. The convertants resulting from gene conversion are distinguished by their tryptophan-prototrophic property. Finally, activity in the forward-mutation system is demonstrated by the occurrence of cycloheximide resistance. Possible mutagenic activity of a test substance is indicated by an increase in the incidence of mutants in comparison with that found in cell suspensions treated with the solvent only.

Results:

In none of the three mutation systems was the incidence of mutants increased by comparison with the negative controls as a result of treatment of the cells with FAT 80 023/A. In the positive controls treated with 4-nitroquinoline-N-oxide, there was a marked increase in the incidence of mutants.

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Conclusion:

Under the conditions of these experiments no evidence of mutagenic effects was obtained.

Classification: Core-Minimum Data

8) Salmonella/Mammalian - Microsome Mutagenicity Test with FAT 80 023/A (Irgasan DP 300) (Ciba-Geigy Ltd; ph 2.632; 78-2511; March 1, 1978)

FAT 80 023/A was tested for mutagenic effects on histidine-auxotrophic mutants (TA92, TA98, TA100, TA1535, TA1537) of Salmonella typhimurium. The investigations were performed with the following concentrations of the test material: Without microsomal activation: 0.01, 0.03, 0.09, 0.27 and 0.81 ug/0.1 ml (Strain TA92: in addition at 2.43 and 7.29 ug/0.1 ml); with microsomal activation: 0.01, 0.03, 0.09, 0.27, 0.81, 2.43 and 7.29 ug/0.1 ml. Positive control experiments were carried out simultaneously.

These tests permit the detection of point mutations in bacteria induced by chemical substances. Any mutagenic effects of the substance are demonstrable on comparison of the numbers of bacteria in the treated and control cultures that have undergone back-mutation to histidine-prototrophism. To ensure that mutagenic effects of metabolites of the test substances formed in mammals would also be detected, experiments were performed in which the cultures were additionally treated with an activation mixture (rat liver microsomes and co-factors).

Results:

In the experiments without microsomal activation, comparison of the numbers of back-mutant colonies in the colonies and the cultures treated with the various concentrations of test material revealed a marked reduction in the colony count due to a growthinhibiting effect of the compound at the concentrations of 0.09 ug/0.1 ml and above. In the experiments performed with microsomal activation, only the highest concentration of the compound caused a reduction in the colony count.

Conclusion:

No evidence of the induction of point mutations by the test material or by the metabolites of the substance formed as a result of microsomal activation was detectable in the strains of S. typhimurium used in these experiments.

Classification: Core-Minimum Data

9) Intrasanguine Host-Mediated Assay with S. typhimurium with FAT 80 023/A (Irgasan DP 300) (Ciba-Geigy Ltd. GU 2.3; March 27, 1979; 78/2803)

FAT 80 023/A was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) in an intrasanguine host-mediated system in male mice. Preliminary experiments were carried out, in which dosages of 0.5, 5, 25, 50 and

100 mg/kg, administered three times by gavage, were tested initially. The main experiments were performed with the doses of 50, 100, 200, and 400 mg/kg, administered three times by gavage.

This test system permits the demonstration of retrograde point mutations in bacteria within the mammalian organism by chemical substances. The peculiarity of the test strains employed consists in a mutation in the hisitidine operon, manifesting itself in histidine-auxotrophism. Mutagenic effects are expressed in a back-mutation to the so-called wild type displaying histidine-prototrophism. The induction of mutations is investigated in the host-mediated assay, on the one hand because the intact animal has the ability to detoxify and eliminate potentially mutagenic substances and, on the other hand, to determine whether substances acquire mutagenic properties through metabolic transformation. In the intrasanguine host-mediated assay, metabolic processes that take place outside the liver also come into play. The potential mutagenic effects of a substance are detected by comparing the frequencies of bacteria that have undergone back-mutation to histidine-prototrophism in the treated animals and the controls.

Results:

Comparison of the results obtained in the dosage groups and the control group revealed no significnat increase in the mutation rates of strains TA98, TA100, TA1535 and TA1537.

Conclusion:

In this test system, under the conditions described, the test material displayed no mutagenic activity.

Classification: Core-Minimum Data

10) Point Mutation Assay with Mouse Lymphoma Cells. I. In-vitro test. II. Host-Mediated Assay (Ciba-Geigy Ltd.; GU 2.3; May 10, 1978; 78-2305, 78-2306)

FAT 80 023/A was tested for mutagenic effects on mouse lymphoma cells (L5178Y) in vitro and in a mouse host-mediated system. The investigations were performed in vitro with concentrations of 15.8 and 28.9 ug/ml and in vivo with the dose of 1313 mg/kg.

These test systems permit the detection of forward point mutations in mammalian cells induced by chemical substances. The gene changes are detected by three marker genes that are contained in the L5178Y-line of mouse lymphoma cells. Mutagenic effects manifest themselves in the occurrence of mutants, expressed by resistance to any of three antimetabolites (methotrexate, cytosine arabinoside and thymidine). Any mutagenic effects of the substances are demonstrable on comparison of the number of clones in the treated and control cultures or in the treated and control animals. Since the intact animal may posses the ability to detoxify and eliminate the potentially mutagenic compound, or alternatively the

the effects of the compound in vivo to be drawn from its effects

in vitro at the cellular level. In the vitro tests, concentrations producing an 80% cell-kill were used. In the host-mediated assay a dosage leading to a decrease of 50% in the number of target cells was administered.

Results:

In the experiments performed in vitro and in the host-mediated assay with dose-levels mentioned above, no increase in the number of mutant colonies was found in comparison with the respective controls.

Conclusions:

In these test systems, under the conditions described, the test material displayed no mutagenic activity.

Classification: Core-Minimum Data

11) Nucleus Anomaly Test in Somatic Interphase Nuclei: Long-term Study with FAT 80 023/A in Chinese Hamster (Ciba-Geigy Ltd.; GU 2.3; August 23, 1978; 78-3005)

The test material, Irgasan DO 300, was administered by gavage at dosages of 75, 150, 300 and 600 mg/kg to Chinese hamsters. The animals were given the substance three times a week for twelve weeks. Six hours after the last dose of the substance the animals were killed. From the bone marrow, smears were made.

The experiment was performed to evaluate any mutagenic effect on somatic interphase cell in vivo. Mutagenic effects present themselves in interphase cells in the form of nucleus anomalies of bone marrow cells. These anomalies occur in interphase cells as a consequence of damage during the mitotic process. The increase in anomalies shows clear dose dependency, comparable to the occurrence of chromosome aberrations in metaphase preparations.

Results:

The bone marrow smears from animals treated with various doses of test material showed no significant difference from the control. The incidence of bone marrow cells with anomalies of nuclei corresponds to the frequency observed in the control group.

Conclusion:

It is concluded that under the conditions of this experiment, no evidence of mutagenic effects was obtained in Chinese hamsters treated with the test material.

Classification: Core-Minimum Data

12) Chromosome Studies in Somatic Cells: Long-term Study with FAT 80 023/A in Chinese Hamsters.

The animals were given Irgasan DP 300 by gavage at dosages of 75, 150, 300 and 600 mg/kg three times a week for 12 weeks. Two hours after the last dose of the test material, colcemide was injected intraperitoneally at a dose of 10 mg/kg and four hours later the animals were killed. From the bone marrow, chromosome preparations were made. The experiment was performed to evaluate any mutagenic effect on the somatic cells in vivo as expressed by chromatid-type and chromosome-type aberrations.

Results:

The chromosome displays showed no specific aberrations in the negative control group, the 75 mg/kg group and the 150 mg/kg group. In one animal each from the 300 mg/kg group and the 600 mg/kg group, one metaphase, i.e. one of 400 in each case, showing a chromatid-type aberration in the form of a chromatid break was found. The incidence of these changes in the 300 mg/kg group and in the 600 mg/kg group was not significantly different from that recorded in the simultaneously examined control group, nor from that recorded in a cumulative control series consisting of 1800 metaphases inspected in long-term studies of the same type. This incidence of aberrations is within the frequency observed in animals of the breed used and considered spontaneous in origin.

Conclusion:

It is concluded that under the conditions of this experiment, no evidence of mutagenic effect was obtained in the Chinese hamster treated with the test material.

Classification: Core-Minimum Data

13) Chromosome studies in male germinal epithelium with FAT 80 023/A (Irgasan DP 300) in the mouse (CIba-Geigy Ltd.; GU 2.3; December 1, 1978; 78-2903)

The test material was administered by stomach tube once daily on each of five consecutive days at dosages of 189, 378 and 756 mg/kg. The mice were killed on the fifth day after the first dose, three hours after receiving an intraperitoneal injection of 10 mg/kg of colcemide, and drop preparations were made of the testicular parenchyma. The investigations were performed to detect any mutagenic effects of the test material on the germinal epithelium, and in particular on the spermatogonia. It has been proved that mutagenic activity can be demonstrated by chromosomal examination of spermatogonia.

Results:

One hundred metaphase plates from each of six mice in the control group and from each of six mice in the groups treated with the low, intermediate and high doses of the substance were examined. In the high-dose group, one chromosometype aberration in the form of a minute was observed in one metaphase from one animal. Comparison of the results obtained in the high-dose group (756 mg/kg) with those found in the concurrently studied negative controls revealed no statistically significant difference.

Conclusion

It can be concluded from these results that the test material exerted no mutagenic action in the mouse under these experimental conditions.

Classification: Core-Minimum Data

14) Chromosome Studies in Male Germinal Epithelium with FAT 80 023/A in the Mouse (Ciba-Geigy Ltd.; GU 2.3; February 23, 1979)

The test material was administered intermittenly by stomach tube at dosages of 189, 378 and 756 mg/kg over a period of 10 days, a single daily dose being given on days 0, 2, 3, 5, and 9. Three days after the final dose and three hours after receiving an intraperitoneal injection of 10 mg/kg of colcemide, the animals were killed. Drop-preparations were made of the testicular parenchyma.

The investigations were performed to detect any mutagenic effects of the test material on the germinal epithelium, and in particular the spermatocytes. It has been proved that mutagenic activity can be demonstrated by chromosomal examination of spermatocytes.

Results:

In all six animals in the control group and in each of the groups treated with the doses of 189 mg/kg, 378 mg/kg, and 756 mg/kg, respectively, 100 metaphase plates each of the primary and secondary spermatocytes were examined. In one animal each from the control group and from the three dosage groups, one spermatocyte I metaphase, i.e., one out of 600 in each case, showing an aberration in the form of a fragment was found. Additionally in one animal from the low-dose group, one spermatocyte II metaphase out of 600 examined metaphase also displayed an aberration in the form of a fragment.

The incidence of the changes in the treated groups was not significantly different from that recorded in the simultaneously examined control group, nor from that recorded in a cumulative control series consisting of 18,600 metaphase figures each of primary and secondary spermatocytes.

Conclusion:

It can be concluded that under the experimental conditions, no evidence of mutagenic effects was obtained on spermatocytes of the mouse.

Classification: Core-Minimum Data

15) Mutagenic Effects of "Irgasan" on Drosophila melanogaster (J. Magnusson; brief report; March, 1979)

An initial toxicity test with and without PCB metabolic enzyme induction was conducted in order to determine a suitable experimental dosage. Different experimental groups of Drosophila males were separately treated with 1000 ppm of triclosan prepared in 3 different vehicles, namely sucrose solution (for drinking), Ringer's solution

(for injection), and corn-oil-ajar (as a food substrate).

The sucrose solution was offered to liquid-deprived male flies for 24-hours while the food preparation was administered for 7-days. Flies treated with the sucrose or Ringer's solution were analyzed to determine their whole body tricolsan content at 0, 24, 48 and 72 (sucrose only) hours post-treatment. Mutagenicity was evaluated using standard techniques to determine sex-linked recessive lethal induction potential in Drosophila males (wild type strain Karsnas 60). Three brood groups were studied: 0-3, 4-6, and 7-10 days post-treatment with new virgin females being given to each male for each brood.

Results:

No toxic effects could be observed at any of the 5 doses tested with or without addition of PCB. A dosage of 1000 ppm was thereby selected for the analytical and mutagenic experiments. Triclosan was found to be excreted very rapidly (within 48-72 hours), whether administered in a drinking solution or in feed, and thus not to pose a cumulative risk. Levels found in the flies immediately after treatment were approximately 9 ppm which then rapidly decreased over 48-hours.

Approximately 15,500 total chromosomes were tested in the sucrose and corn oil triclosan experiments. No indication of increased mutagenic induction was observed after triclosan treatment.

Conclusion:

There is no indication of any mutagenic effect by Irgasan in Drosophilia as judged by the present experimental series on sex linked recessive lethals.

Classification: Core-Minimum Data

16) Nephrotoxic and Hepatotoxic Effects of Triclosan and Chlorhexidine in Rats (Chow, A.Y.K., Hirsch, G.H., and Buttar, H.S. (1977). Toxicol. Appl. Pharmacol. 42, 1-10); Authors' Abstract

The in vivo and in vitro effect of pretreatment with triclosan and chlorhexidine on the accumulation of p-aminohippurate (PAH) and N-methylnicotinamide (NMN) by male rat kidney was studied. Adult rats pretreated orally with triclosan between 0.625 and 2.5 gm/kg for 24 hr. showed a dose-related inhibition in the accumulation of PAH but not NMN. The maximal inhibitory effect from 2.5 gm/kg of triclosan was observed between 12 and 24 hr. In vitro addition of 10^{-5} or 10^{-4} M triclosan inhibited both PAH and NMN accumulation by kidney slices prepared from immature (1 to 6 weeks old) as well as adult rats. In contrast, chlorhexidine produced a dose-related inhibition of both PAH and NMN accumulation in vitro and in vivo. Maximal inhibition of PAH and NMN accumulation was observed between 24 and 48 hr. after chlorhexidine (2.0 gm/kg) treatment.

Blood urea nitrogen (BUN) and plasma transaminase, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase) activities were elevated 24 hr. after administration of 1.0 gm/kg or higher doses of chlorhexidine but not after pretreatment with triclosan. These results suggest that both triclosan and chlorhexidine affect renal function as indicated by the accumulation of PAH and/or NMN, but that only chlorhexidine is hepatotoxic.

Classification: Core-Minimum Data

B. Studies Submitted on June 11, 1979

1. 90-Day Bathing of Newborn Rhesus Monkey with Triclosan Soap Solution (Hazelton; April 26, 1979; LH17857 E-F, LH858 C-D)

Ten newborn rhesus monkeys were bathed 5 minutes daily for 90 consecutive days in a liquid soap solution containing 0.1% triclosan while a second group of 10 monkey infants were bathed in the soap solution devoid of Triclosan. Daily, seven days per week for 90 days, each animal was held over a sink and the trunk sponged down with warm water. Fifteen(15) ml of the test material was applied by syringe to the trunk (excluding head, tail and extremities) and lathered for 5 minute periods. Care was taken to prevent the animal from ingesting the test materials during bathing. The animals was then held under flowing warm water until all evidence of the soap was removed. The animal was then thoroughly dried with a large bath towel and placed in a warm isolette. The time of bathing was recorded. One-half of the animals in both the test and control groups were necropsied at the end of the 90-day bathing period while the balance of the animals were held for an additional 30 days without bathing and then necropsied.

Daily observations relating to general health status and nursery care procedures were recorded. These include body weights, formula consumed, abnormal stools and deviations from general health status.

Three(3) ml of heparinized blood for Triclosan analysis and sufficient clotted blood (2.0 ml) for total and direct bilirubin was collected on day 1 from half the animals in each group and on day 2 from the remaining animals. In addition, all animals were bled on days 15, 30, 45, 60, 75 and 90. The recovery animals were also bled on days 104 and 118.

On days 30, 60, 90, 104 and 118, blood was also collected for hematology (RBC, WBC, differential WBC, hematocrit, hemoglobin and reticulocyte count), and serum chemistry (BUN, creatinine, SAP, SGOT, SGPT, glucose, Na^+, K^+, Cl^-).

Animals were placed in metabolism cages for 24 hours at 30, 60, and 90 days and on day 118 for the recovery animals. They were removed from the metabolism cage, bathed and returned. Urine was directed from the metabolism cage to an alcohol-dry ice cooled collection bottle. Feces was frozen upon removal. All specimens were sent to the sponsor for Triclosan analysis.

Random samples of the test and control solutions were returned to the sponsor at monthly intervals for Triclosan analysis. At the conclusion of the study, unused portions of the materials were returned. Groups 1B and 2B were necropsied at the end of the 90-day bathing period while groups 1A and 2A were necropsied after an additional 30-day holding period during which bathing was

discontinued. Animals were given a lethal dose of sodium pentobarbital and exsanguinated.

A gross pathologic examination was performed on each monkey at sacrifice and brain, pituitary, thyroid, heart, liver, kidney, adrenal, thymus and gonad weights obtained. Microscopic examination of 35 tissues or organs was also carried out on each monkey. Portions of the following organs were also obtained for Triclosan analysis: eye, skin (treated area), skin (untreated area), spleen, adrenal gland, liver, testes, ovaries, thymus, lung/trachea, heart, thyroid, skeletal muscle, sciatic nerve, bone marrow, brain and fat.

Results:

The study went essentially as planned with few problems or changes. No deaths were encountered. Four animlas experienced mild transient illness but these were not related to compound administration. There were no compound-induced abnormalities noted grossly or upon histologic examination of the tissues.

Body weight progressions were well within normal ranges for both control and test animals. Based on the lack of any detectable differences between control and treated groups, it would appear that daily bathing in soap containing the Triclosan did not induce any alterations in hematology or serum chemistry which were not also seen in those animals bathed in soap devoid of Triclosan.

Complete necropsies performed on all animals at the conclusion of the study revealed no gross evidence of compound-related changes. Organ/body weight ratios for control and test animals appear similar but were not evaluated statistically due to the small number of animals involved. Application of Triclosan under the conditions of the experiment resulted in no histomorphologic findings that were attributable to the compound.

Conclusion:

The area of the animals exposed to treatment (the trunk) is less than the area of exposure during washing for a human infant. The area and time of dermal exposure (5 minutes/day) may not be appropriate for extrapolation to washed human infants. In addition, since the Triclosan concentration (0.1%) used in the study is the same concentration used to bathe human infants, the margin of safety for human infants is not apparent.

Classification: Supplementary Data

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2. Irgasan DP 300 90-Day Bathing Study in Newborn Rhesus Monkeys. Final Analytical Report (Ciba-Geigy; June 4, 1979)

Ten infant rhesus monkeys were bathed daily from birth through day 90 with 15 ml of a soap solution containing 0.1% of Irgasan DP 300. The Irgasan DP 300 in the soap solution was non-radioactive.

The soap solution was applied to the trunk area of each animal, lathered for a five minute period, and then rinsed off with warm running water. Ten control animals were treated in the same manner with a soap solution containing no Irgasan DP 300. After the 90-day treatment period, 5 control animals and 5 treated animals were sacrificed. The remaining animals were kept without bathing for a subsequent 30-day recovery period. Blood samples were collected from each animal on days 15, 30, 45, 60, 75, and 90. In addition, blood samples were collected from one-half of the monkeys at day 1 and from the other half at day 2. Recovery group animals also had blood samples taken on days 105 and 120. Urine and feces samples were collected on days 30, 60, 90 and 120. Tissue samples were taken from animals at sacrifice.

Results:

Blood levels of total Irgasan DP 300 ranged from 0.17-0.97 ppm. Plateu levels were reached within 15 days of treatment. All of the Irgasan DP 300 was present in the conjugated form - either glucuronide or sulfate. The glucuronide conjugate dominated in the initial samples (taken on day 1-2); however, the sulfate conjugate dominant in all subsequent samples. Blood samples taken on day 90 contained almost exclusively Irgasan DP 300 sulfate (80-90% of the total present). No Irgasan DP 300 was detected in blood from the control animals or in blood taken from the treated animals during the recovery phase. The latter indicates that Irgasan DP 300 was rapidly eliminated when treatment ceased.

Urinary concentrations of Irgasan DP 300 ranged from 0.3-4.8 ppm during the treatment period and fell to trace levels during the recovery period. Some free Irgasan DP 300 was detected; however, the majority of the Irgasan DP 300 was present as the glucuronide conjugate.

Fecal concentrations of Irgasan DP 300 ranged from < 0.1-10.5 ppm during the treatment period and dropped to trace levels during the recovery period.

Essentially all of the Irgasan DP 300 was present in the free (unconjugated) form. Experiments showed that baby monkey urine is capable of hydrolyzing Irgasan DP 300 glucuronide and that baby monkey feces is capable of hydrolyzing Irgasan DP 300 sulfate. Therefore, the free Irgasan DP 300 found in these two matrices may have resulted from hydrolysis in vivo or in vitro (i.e., during analysis).

Small amounts of Irgasan DP 300 were detected in tissues from treated animals sacrificed 1-5 days after the 90-day treatment period. Highest concentrations were present in lung (< 0.1-1.3 ppm), liver (< 0.1-0.5 ppm), kidney (< 0.1-0.9 ppm), and skin (0.2-1.9 ppm) Irgasan DP 300 was detected in skin samples from animals sacrificed after recovery period. None was detected in the other tissues.

Soap solutions were monitored at montly intervals throughout the study. The Irgasan DP 300 content of all the soap solutions was within 10% of the nominal concentration.

Conclusion:

Irgasan DP 300 is dermally absorbed, as evidenced by the systemic concentrations in blood and tissues. Excretion of Irgasan DP 300 occurs fairly rapidly, however the potential for bioaccumulation should be considered, although low, from the fact that blood levels plateau at 15 days, transfer to tissues is rapid and tissue residues are present after continued washing. No tissue residues (except skin) were present 30 days after treatment.

Classification: Supplementary Data

(a) Only one dose level tested.

3. Pilot Study. Single Dose Dermal Absorption of Triclosan in 3-Days Old Rhesus Monkeys. Analyses of blood and soap samples (Ciba-Geigy; 6/5/78)

Two rhesus monkeys were washed with a soap solution containing 0.1% Triclosan at 3 days of age. Blood samples were taken at 1, 3, 5, 8, 12 and 24 hours after washing.

Results:

The amount of conjugated Triclosan (both glucuromide and sulfate) found in these samples ranged from 0.25-0.68 ppm. No free, unconjugated Triclosan was detected. The plateau level, reached by 8-12 hours after washing, was maintained at the 24 hr. period. Glucuronide and sulfate ratios varies throughout this period.

Classification: Supplementary Data

(a) Pilot Study only.

4. Irgasan DP 300 Oral Dose Kinetic Study in Adult Rhesus Monkeys (Ciba-Geigy; 10/19/78)

Four adult rhesus monkeys (3 males and 1 female) were given a single oral dose of Irgasan DP 300 (5 mg/kg). Blood samples taken taken from the animals prior to dosing and then 1 hr., 2 hr., 3 hr., 5 hr., 8 hr., 12 hr., 2 days, 3 days, 5 days, 7 days and 14 days after dosing. Twenty-four hour composite urine and feces samples were collected on days 1-5.

Results:

The concentration of Irgasan DP 300 in blood increased rapidly after dosing and reached a peak level within 3 to 5 hours. The peak level ranged from 1.9-3.9 ppm. After the peak level was attained, the Irgasan DP 300 concentration in blood gradually decreased and reached non-detectable levels 5-7 days later.

Irgasan DP 300 in blood existed in conjugated form-either sulfate or glucuronide. Glucuronide and sulfate ratios varied with time.

Most of the Irgasan DP 300 was excreted in the urine and feces within 3 days after dosing. Urinary excretion dominated over fecal excretion in all but one animal. Feces contained primarily free (unconjugated) DP 300 while urine contained primarily glucuronide conjugated Irgasan DP 300. From 83-98% of the administered Irgasan DP 300 was accounted for in the excrement from the animals.

Classification: Supplementary Data

(a) No tissue analyses were done.

5. Pharmacokinetics of Triclosan in Rat after Intraveous and Intravaginal Administration (W.H. Siddiqui and H.S. Buttan; J. Environmetal Pathology and Toxicology, 2:861-871 (1979) Authors' Abstract

The pharmacokinetics of triclosan was studied in sexually mature virgin Wistar rats. C^{14}-triclosan was injected either via the femoral vein (i.v., 5 mg/kg in polyethylene glycol-400) or into the vaginal orifice (ivg, 5 mg/kg in corn oil). Radioactivity was determined by liquid scintillation spectrophotometry.

After iv administration, the disappearance of C^{14} from the blood followed the kinetics of a two-compartment open-system model. The apparent volume of distribution was 42 percent of the body weight, which is more than the extracellular water, suggesting a rapid transfer of this antimicrobial agent from plasma to tissues. The blood half-life of C^{14} during the B-phase was 8.8 $\pm$ 0.6 hr. and the blood clearance rate was 77.5 $\pm$ 11.3 ml/kg/hr. After ivg administration of C^{14}-triclosan, the radioactivity was detected in tail blood at 15 min., peaked between 2 to 4 hr., and declined rapidly to 6 hr. and more slowly thereafter. About 26 percent of the administered dose remained in the vagina after 4 hr. and 12 percent after 24 hours. Tissue concentrations of C^{14} were highest in the plasma, kidney and liver after ivg application, but extremely low in the brain, fat and skeletal muscle. The percentage of the dose excreted in 24 hr. in the feces and urine were 18 and 9 after iv administration, compared to 26 and 14 after the ivg route, respectively. The results show that triclosan is rapidly absorbed through the vaginal mucosa of the rat.

Classification: Core-Minimum Data

6. Metabolism of Chlorodiphenyl Ethers and Irgasan DP 300 (M. Th. M. Tulp, G. Sundstrom, L.B.J.M. Martron and O. Hutzinger, Xenobiotica, 1979, Vol. 9, No. 2, pp. 65-77); Authors' Abstract

1. In the rat chlorodiphenyl ethers are metabolized via two routes. The predominant reaction is aromatic hydroxylation; scission of the ether bond is a minor metabolic process.

2. In all cases, primary hydroxylation takes place ortho and meta to the ether bond. Ortho-hydroxylation leads to the formation of "predioxins" in cases where the parent compounds contain a chlorine atom in one of the ortho positions in the second ring.

3. 5-chloro-2-(2,4-dichlorophenoxy) phenol (Irgasan DP 300), a compound that meets the structural requirements of a predioxin, did not yield chlorodibenzodioxins or hydroxylated derivatives thereof.

4. Irgasan DP 300 is excreted unchanged in feces and urine (partly conjugated) but is also hydroxylated to five different monohydroxy metabolites which were found in urine; three of these were also present in feces. As a result of scission of the ether bond, 2,4-dichlorophenol occurred in urine and feces and 4-chlorocatechol in urine.

5. Neither in the case of Irgasan DP 300, nor in that of chlorodiphenyl ethers with an ortho chlorine atom, could metabolic cyclization to chlorodibenzofurans or their hydroxylated derivations be detected.

Classification: Core-Minimum Data

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