Hepatotoxicity of a Series of Organotin Esters

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Four organotin esters having use or potential use in the formulation of medically used plastics were evaluated for their toxic effects on the liver following oral administration. The liver damage is apparently the cumulative type, requiring several days to manifest damage in the form of elevated SGPT values and prolonged hexobarbital narcosis. The toxic potential was in general solubility related.

CERTAIN ORGANO-METALLIC compounds are currently being used as stabilizers in plastic formulations, curing agents for rubber formulations, antifungal agents, and many other industrial applications. The bulk of the toxicity investigations to date on organotin compounds have been conducted with the alkyl–tin salts of the general formula R—Sn—X, R being an alkyl group, Sn as quadrivalent tin, and X representing a halide, sulfate, or other salt-forming ion. An excellent paper by Barnes and Stoner (1) reviews many of the toxic aspects of these compounds. Relatively little has been published concerning the toxicity of the alkyl–tin esters, R—Sn—O—R, particularly with a view toward delineating the relationship which may exist between varying the number of alkyl groups and the oral toxicities of these compounds. In addition, certain differences have been noted by Barnes and Stoner as to species variations when using the salts of alkyl–tin compounds.

In view of the greater interest being shown in certain of the alkyl–tin esters for various uses in devices for medical and pharmaceutical applications, it was decided that an investigation of the acute oral toxicity of a series of organotin compounds be undertaken. The esters chosen for this study were the acetate esters of the di- and tributyl compounds. In addition the tetrabutyl compound was selected which naturally has no point in the molecule available for esterification. A fourth compound, dibutyltin di-(2-ethylhexoate), was included as a check for the effect of the ester moiety on the resulting toxicity.

EXPERIMENTAL

Materials.—The materials used in this study were: (*a*) tetrabutyltin, lot 42921; (*b*) tributyltin acetate, lot GPI-1281; (*c*) dibutyltin diacetate, lot GP-1108; (*d*) dibutyltin di-(2-ethylhexoate), lot 1246.

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Methods

Oral LD_{50} Determinations.—Since published data were not available, it was necessary to establish LD_{50} values and time of onset of toxicity. These values were determined by a single oral intubation of each organotin, dissolved in sesame oil, into groups of 10 male Swiss-Webster albino mice of uniform weight and age, which had been deprived of food for 24 hr. Dosage levels of organotin were spaced in geometric progression increasing by a factor of two. The volume of each dose was 0.5 ml./20 Gm. body weight. Following intubation, observations for mortality were recorded daily for 1 week.

Liver Function Tests.—Previous reports in the literature have indicated that certain organotins exert an hepatotoxic effect (2), while others indicate toxicity to the bile duct (3). A series of experiments was undertaken to define more clearly the hepatotoxicity of the tin esters. Rabbits were used to obtain direct and indirect evidence of acute toxicity to liver tissue. They were intubated with a single oral dose of each organotin, and the following liver function tests were performed.

(*a*) Serum glutamic-pyruvic transaminase (SGPT), as an index of hepatocellular injury. (*b*) Prothrombin time, to indicate liver damage in the absence of jaundice. (*c*) Alkaline phosphatase, as an early indicator of hepatic obstruction which might lead to jaundice. (*d*) Thymol turbidity, to differentiate cellular from obstructive hepatic disease.

Four groups of rabbits, three rabbits per group, were each administered approximately one-half the LD_{50} dose of each organotin. A fifth group, administered only the sesame oil vehicle, served as controls. Values obtained from each rabbit before organotin dosing also served as controls for determining acute response after dosing. Five-milliliter blood samples were withdrawn from the median ear artery before dosing and again at intervals of 48 and 144 hr. (6 days) after dosing.

Cholestasis in Mice.—Plaa and Becker (4) recently reported a reliable method for the determination of intrahepatic cholestasis. This method employs an intravenous dose of fluorescein dye, which accumulates in the gall bladder and bile duct in the absence of intrahepatic bile stasis. The dye is visualized under ultraviolet light as a brilliant yellow-green color. If cholestasis does occur due to a toxic response or liver damage, fluorescence in the biliary tract should be absent, using all-or-none criteria. This method was used in this study. Groups of 10 mice were orally intubated with 50 mg./Kg. of organotin (except dibutyltin diacetate at 25 mg./Kg.). After an interval of 7 days the fluorescein dye was injected intravenously *via* the

tail vein, 0.1 ml./10 Gm., with a concentration of 20 mg./10 ml. in 0.17 *N* $NaHCO_3$. Fifteen minutes later the animals were sacrificed and examined under ultraviolet light for yellow-green fluorescence.

Determination of Blood Concentration of Tin.—To prove gastrointestinal absorption, hence blood passage through the liver, it was necessary to determine blood levels of tin at various time intervals following oral intubation. Five-milliliter samples of blood were widthdrawn by cardiac puncture from New Zealand albino rabbits prior to a single intubation with one-half the LD_{50} dose of organotin. These blood samples were then assayed for presence of tin according to the method described by Feigl (5). Additional 5-ml. samples were taken at intervals of 1, 3, 5, and 18 hr. following organotin intubation, and similarly analyzed.

Hexobarbital Narcosis.—This study was conducted to determine (*a*) a direct CNS effect after a relatively short time lapse following a single oral dose of organotin and (*b*) the presence of liver damage or change in function after a relatively long period of time following a single oral organotin administration. For part (*a*) sodium hexobarbital, 60 mg./Kg., was administered intraperitoneally 1 hr. after organotin dosing, and in part (*b*) the sodium hexobarbital was administered 7 days after organotin dosing. In both cases one-fourth the LD_{50} dose was given. Sleeping time of dosed mice in parts (*a*) and (*b*) were compared with that of concurrent controls. Sixteen albino male mice per test group were used.

Pathology.—Liver and kidney tissues from animals exposed on an acute and subacute basis to doses of organotins were obtained immediately after sacrifice and preserved in buffered formalin solution. These were subsequently stained with the standard hematoxylin-eosin stain and examined for changes which might be attributed to the organotin compounds.

Response to Acute and Subacute Administration.—In order to broaden the toxicity profile on the compounds under study for effects on the liver, and perhaps to more accurately correlate data, some general observations were made on animals dosed on an acute and subacute basis. Consequently, one group of animals was administered oil solutions in smaller doses, but repeated at intervals over a 1 to 2-week period. A third group of animals was administered saturated aqueous solutions by the intraperitoneal route for acute observations.

RESULTS AND DISCUSSION

LD_{50} Determination.—The oral administration of toxic concentrations of organotin, as oil solutions, resulted in deaths over a long period of time. The earliest deaths began to appear about 8 to 12 hr. after dosing, but continued over a period of days. For purposes of calculation of LD_{50} values, an arbitrary period of 7 days was set for counting deaths due to organotin poisoning. After the 7-day period, any deaths occurring did not enter the calculations, because it was difficult to determine whether death was directly attributable to the compound or whether it occurred through a secondary depressive starvation for food and water. The results of this study are listed in Table I, with confidence limits calculated by the method of Weil (6) and Thompson and Weil (7). It should be noted that the trialkyl ester (tributyltin acetate) had a slightly higher toxicity than the di-ester, though not significantly so. The tetrabutyltin exhibited a very low order of toxicity. Dibutyltin di-(2-ethylhexoate) had an LD_{50} value approximately twice that of the di- and tributyl acetate esters. These data seemed to indicate that the alkyl moiety had less effect in the determination of death than the ester moiety.

Liver Function Tests in Rabbits.—Following a single oral dose (one-half the LD_{50} dose) of the organotin compounds, only the SGPT values showed any significant change from the normal control values. No significant SGPT elevations at the 48-hr. time interval were noted (Fig. 1). At the 144-hr. interval, however, values of all organotin-dosed rabbits showed significant ($P = 0.05$) increases over control values, except with dibutyltin diacetate. The prothrombin time, alkaline phosphatase, and thymol turbidity showed no significant changes from control values at any of the time periods.

SGPT is a highly specific index of hepatocellular injury and is much more sensitive to minimal or moderate damage to the liver than are the other hepatic function tests. The transaminase activity in blood serum parallels the degree of liver cell injury. Elevation of the SGPT indicates that one of the first hepatic responses to organotin poisoning is

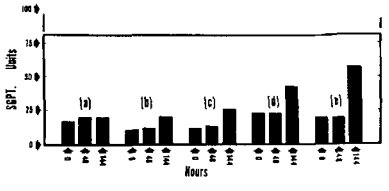

Fig. 1.—Serum glutamic-pyruvic transaminase (SGPT) values in rabbits receiving single oral doses of organotin (average of three rabbits), at 0, 48, and 144-hr. intervals. Key: (a) control rabbits; (b) rabbits receiving dibutyltin diacetate; (c) rabbits receiving dibutyltin di-(2-ethylhexoate); (d) rabbits receiving tributyltin acetate; (e) rabbits receiving tetrabutyltin.

TABLE I.—APPROXIMATE BLOOD LEVELS OF ORGANOTIN 3 hr. AFTER ORAL DOSAGE WITH LD_{50} VALUES

Compd.	Mol. Wt.	Single Oral Dose, mg./Kg.	Elemental Tin in Blood, p.p.m.	Oral LD_{50} Values, mg./Kg. ± S.E.
Dibutyltin diacetate	350.7	110	90	109.7 ± 24.2
Dibutyltin di-(2-ethylhexoate)	518.7	200	130	199.9 ± 43.2
Tributyltin acetate	348.7	300	140	99.1 ± 8.4
Tetrabutyltin	410.7	500	200	6000.0 ± 500.0

from parenchymal cells and involves the activity of one or more enzymes. In the case of the other liver function tests, none proved sensitive enough to detect subtle changes that may have occurred. Lack of any significant change in SGPT values from normal 48 hr. after oral dosing indicated that either the organotin does not reach the site of action in the liver cells in sufficient concentration to produce physiological disturbances of any great consequence within 48 hr., or that the changes produced there are produced slowly so that the accumulated response does not manifest itself until sometime after 48 hr. This also confirms other data indicating a delayed hepatotoxicity from the organotin compounds.

Intrahepatic Cholestasis and Jaundice in Mice.—In the course of the LD_{50} determination, it was noticed that three of the organotin compounds at the higher dose levels (200 mg./Kg.) caused visually evidenced jaundice in mice. Dibutyltin diacetate, tributyltin acetate, and dibutyltin di-(2-ethylhexoate) in large doses produced, in from 5 to 7 days, visually apparent discoloration of the skin, internal organs, and urine. Tetrabutyltin did not cause an apparent jaundiced discoloration in any of the test animals. Confirming evidence of intrahepatic cholestasis by the method of Plaa and Becker (5) is summarized in Table II. This table presents the total number of animals showing cholestasis at various dose levels, all exceeding 50 mg./Kg. These data indicated that all four compounds were at least capable of inducing cholestasis. Since none of the control animals showed cholestasis by the fluorescein dye method, this condition in the other animals must be attributed to the organotin.

Absorption of Organotin into Bloodstream.—Feigl spot tests for tin in the bloodstream of rabbits were negative prior to the oral administration of the four tin compounds. Five-milliliter blood samples taken 1, 3, 5, and 18 hr. after oral intubation of organotins were positive for the presence of tin in all cases. The test color intensity was compared with standards of known concentration. By this comparison it was found that increasing concentrations of tin appeared in the bloodstream, reaching a maximum at 3 hr. after intubation. Positive tests for tin at substantial levels were also obtained through 18 hr., at which time the study was terminated. The blood level of each organotin at the 3-hr. time period was calculated (Table I).

Results of the Feigl spot tests indicated that, with all four compounds, either the organotin itself or a metabolite was capable of being carried into the bloodstream in a reasonably short time in amounts large enough to create a pharmacological or toxicological response. In addition, the presence of relatively high levels of tin persistent in the blood stream after 18 hr. suggested the possibilities that either a large storage depot existed somewhere in the body from which amounts of organotin were steadily being released or that the organotin remained in circulation for a protracted length of time. Concurrent spot tests for tin in the bloodstream of rabbits which had been orally dosed 13 days prior to testing proved negative. This indicated that should a prolonged retention exist, it was depleted within the 13-day period.

Table II.—Cholestasis 1 Week After Oral Organotin Administration

Compd.	Mice, No.	% Showing Cholestasis
Sesame oil controls	17	0.0
Dibutyltin diacetate	33	18.2
Tributyltin acetate	32	6.3
Tetrabutyltin	14	35.7
Dibutyltin di-(2-ethylhexoate)	36	22.2

Table III.—Effect of Organotins on Hexobarbital Narcosis in Mice

Compd.	Mice, No.	Oral Dose	Sleep Time ± S.E., min.	Significance, P
Control group (sesame oil)	14	0.5 ml./20 Gm.	29.0 ± 14	....
Dibutyltin diacetate	12	30 mg./Kg.	44.5 ± 11.9	0.01
Tributyltin acetate	13	50 mg./Kg.	43.1 ± 8.1	0.01
Dibutyltin di-(2-ethylhexoate)	8	40 mg./Kg.	37.7 ± 11.7	0.05

Hexobarbital Narcosis in Mice.—When sodium hexobarbital (60 mg./Kg.) was administered intraperitoneally 1 hr. after oral dosing with the organotins, there was no significant difference between the sleeping times of the test and the control groups of mice. This indicated that within the limitations of the technique, there was no significant influence on the CNS from these compounds within a 1-hr. time span. On the other hand, groups of mice dosed with sodium hexobarbital (60 mg./Kg.) intraperitoneally 1 week after a single dose of each organotin compound showed significant differences from control sleeping times (Table III). The mice received one-fourth LD_{50} doses. There was a significant prolongation of sleeping times among mice receiving dibutyltin diacetate and tributyltin acetate. This indicates that liver damage from these two compounds did occur to some extent by the end of 1 week. This was confirmed by pathologic manifestations in liver sections. The tetrabutyltin is apparently unstable as evidenced by an increasing toxicity with time. The oral LD_{50} value established on fresh material was approximately 6000 mg./Kg., but it was noted in sleeping-time studies that all mice dosed at one-fourth LD_{50} doses died almost immediately. Rechecking of data and materials indicated that the tetrabutyltin compound had decomposed. Therefore, the sleeping-time data for this compound was discarded. All other data reported in this paper were conducted on the fresh material and are therefore included.

Acute Pharmacological Response.—Response to single large oral doses of organotin dissolved in sesame oil produced no sudden or dramatic responses in mice, even in concentrations 5 to 8 times the LD_{50} value, suggesting slow gastric absorption. After 30 min. to 1 hr., a gradual depressive syndrome was noted, in which activity was decreased, respiration rate, and irritability reduced. Almost complete ptosis was observed after 1 to 2 hr. Death at higher dose levels occurred within 18 hr., but at the LD_{50} levels, death occurred over a period of several days.

Saturated aqueous solutions of each organotin compound were prepared in normal saline and injected, 0.5 ml./20 Gm., intraperitoneally in mice.

Two compounds, dibutyltin diacetate and tributyltin acetate, produced a rapid onset of symptoms, those caused by the former being most severe. Writhing and stretching were observed in both cases within 2 to 4 min. Those injected with dibutyltin diacetate showed severe depression, ataxia, and ptosis with death resulting within 30 to 35 min. due to apparent respiratory paralysis. Gasping with the mouth widely opened was a common response. Cyanosis preceded death. These symptoms were observed in mice receiving the tributyltin acetate, but onset was less rapid and severe, with death occurring between 30 to 60 min. Mice injected with tetrabutyltin showed stretching, but symptoms were very mild by comparison with those caused by the two previously mentioned (no deaths resulted). Solutions of dibutyltin di-(2-ethylhexoate) caused little response except depression, and no deaths occurred with this compound.

Subacute Responses.—Rats dosed repeatedly with one-half the LD_{50} dose at 3 to 5-day intervals generally showed little response either immediately after each dose or at the end of a 1-month time period, with the exception of those receiving dibutyltin diacetate. Slight temporary depression was noted with tributyltin acetate. In the case of the dibutyltin diacetate, diarrhea, ptosis, and general depression was noted, with individuals varying in their response. Onset of diarrhea was observed after the first dose, but the animals did not seem to become depressed to any extent until after receiving four to five doses. In these cases little was observed except depression. Death occurred in two of five rats after the fifth and sixth dose (total dose 250 to 300 mg./Kg.).

Rabbits dosed repeatedly with the same levels of dibutyltin diacetate tended to exhibit diarrhea to a greater extent than rats receiving the same dose. In one rabbit, following six repeated doses with dibutyltin diacetate at weekly intervals, complete paralysis of the hind legs resulted. Dosing was thereupon discontinued and no recovery was seen after 3 weeks. Other rabbits exhibited considerable malaise and some depression, but no paralysis was noted after seven doses.

Pathology.—The tissue sections submitted for pathological examination generally confirmed that the organotin compounds do exert a toxic effect on liver tissue. A significant finding is that liver damage is rather slowly manifested after a single oral dose. No pathology of liver tissue could be observed 3 days after a single dose of organotin, but definite pathology could be observed after 7 days. The primary change in liver tissue of rats and mice appears to be a moderate increase in reticuloendothelial cells and mild hepatocellular damage with cloudy swelling, which could cause retention of bile. (See Fig. 2.)

These data confirm prior indications of liver damage at the 7-day time interval as evidenced by the prolonged sleeping time from hexobarbital, by the elevated SGPT, by bile stasis indicated in the fluorescein test, and by jaundice from high doses in mice. Apparently there was no bile duct pathology during this time period as had been observed for salts of certain organotin compounds (1). This was somewhat surprising in view of the elimination of these compounds by way of the bile into the intestinal tract and finally through the feces.

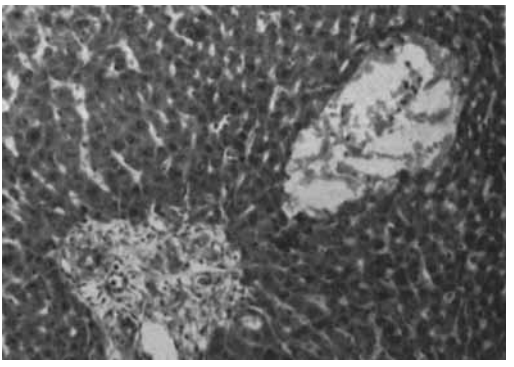

Fig. 2.—Liver of rat receiving dibutyltin diacetate, nine repeated oral doses of 50 mg./Kg., spaced over a 5-week period. The tissue shows cloudy swelling, most noticeable in the centrilobular location, with minimal fatty degeneration. A slight increase in fibrous connective tissue exists, and there are islands of acute necrosis, with minimal infiltration of polymorphonuclear leucocytes throughout the section.

SUMMARY

The results of the experiments on a series of organotin esters indicated that the compounds are absorbed from the gastrointestinal tract following oral administration in an oil solution. After absorption into the bloodstream, there is evidence that the compounds remain in the blood for periods in excess of 18 hr. and then are eliminated *via* the feces. In the passage of these compounds through the liver, there accumulates over a period of several days a uniform and general liver damage. This damage usually takes the form of cloudy swelling, mild hepatocellular involvement, and a moderate increase in reticuloendothelial cells following a single oral dose. There does not appear to be any significant difference between the hepatotoxic effects of the di- and tributyl esters, but the tetrabutyl compound exhibits a reduced toxicity. In view of the delayed effects of these compounds on liver tissue, it is suggested that long-term studies should be conducted on them.

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