Brit. J. Pharmacol. (1955), **10**, 16

STUDIES ON THE TOXICITY OF ALKYL TIN COMPOUNDS

BY

H. B. STONER, J. M. BARNES, AND JANET I. DUFF

From the Medical Research Council, Toxicology Research Unit, Serum Research Institute, Carshalton, Surrey

(RECEIVED JULY 8, 1954)

In recent years the toxicity of tin has aroused little interest, but in the latter part of the last century and the first decade of this, considerable attention was paid to the possible contamination of food preserved in cans (White, 1881; Ungar and Bödlander, 1886; Lehmann, 1902; Eckhardt, 1909; Schryver, 1909). It was concluded that there was no serious risk; since then there have only been occasional studies on the toxicity of tin compounds (Salant, Rieger, and Trenthardt, 1914; Handovsky, 1925; Schwartzer and Clarke, 1927; Seifter and Rambousek, 1943). A suggestion that the fungicidal properties of certain organic tin compounds might have industrial application (Van der Kerk and Luijten, 1954) coupled with their use as "plasticizers" has rekindled interest in this subject. In most reference books the toxicity of tin tends to be discounted, but preliminary work with its alkyl derivatives showed that on a molar basis tin was five times as toxic as beryllium. Its potentialities have been underestimated because of the difficulty of introducing it into the body in a soluble form. The full toxicity is only seen when the alkyl derivatives are used, some of which are volatile and soluble in water at *p*H 7. A number of these and related compounds have now been examined. Many of their toxic properties are already well outlined (White, 1881; Ungar and Bödlander, 1886), but little attention has been paid to them recently (Seifter, 1939a; McCombie and Saunders, 1947) and it is now possible to extend these findings. Later knowledge, particularly regarding their action on the central nervous system, has an important bearing on the assessment of the possible hazards to health during the production and use of the alkyl tin compounds.

METHODS

Experiments were carried out on albino rats fed on M.R.C. diet 41 (Bruce and Parkes, 1949); on rabbits, guinea-pigs, and domestic fowls fed on M.R.C. diet 18 (Bruce, 1947), and on cats. Cabbage was added to the diet of the guinea-pigs. The tin compounds used, with their chemical properties, sources, and method of administration, are shown in Table I.

The cardiovascular effects of these compounds were observed in the cat and rat under pentobarbitone sodium anaesthesia (50 mg./kg. body wt. i.p.) and in the rabbit under urethane (4.0–5.0 ml. 25% w/v solution/kg. i.v.). The blood pressure was recorded from the carotid artery with a mercury manometer. Rectal and skin temperatures were determined with a thermocouple.

TABLE I

TIN COMPOUNDS USED, WITH THEIR VEHICLES AND ROUTES OF ADMINISTRATION

Compound	Vehicle	Route
*Tetraethyl tin	Nil	i.v., i.p.
*Trimethyl tin sulphate	0·5% (w/v) in 0·9% (w/v) NaCl, *p*H 7·4	i.p., i.v.
*Triethyl tin sulphate	0·5% in 0·9% NaCl, *p*H 7·4	i.p., i.v., p.o.
* " " "	1% in arachis oil	p.o.
* " " "	6% in dimethyl phthalate	p.c.
*Triethyl tin hydroxide	0·5% in arachis oil	p.o.
*Tri-*n*-propyl tin acetate	1% " " "	"
*Tri-*iso*-propyl tin acetate	1% " " "	"
Tri-*n*-butyl tin acetate	2% " " "	"
" " "	0·5%, 2% in Tween 80	i.p.
*Tri-*n*-hexyl tin acetate	2% in arachis oil	p.o.
*Triphenyl tin acetate	2·5% and 4% in arachis oil	p.o., i.p.
*Diethyl phenyl tin acetate	2·5% in arachis oil	p.o.
*Diethyl tin dichloride	2% in Tween 80	i.p.
* " " "	2% in arachis oil	p.o.
* " " "	2% in propylene glycol	i.v.
*Diethyl tin diiodide	2% in Tween 80	i.p.
* " " "	2% in arachis oil	p.o.
*Dibutyl tin dichloride	1% " " "	"
*Dibutyl tin dilaurate	Suspension in arachis oil	"
*Monoethyl tin trichloride	2% in water neut. *p*H 5–6	i.v., i.p.
Stannic chloride (B.D.H.)	0·4% in arachis oil	i.p.
Stannic sodium chloride (B.D.H.)	0·7% in water *p*H 2·3	i.v.
†Stannic lactate	1·3% " " *p*H 7·0	"
‡Stannic citrate	4% " " *p*H 7·0	"
§Sodium stannitartrate (34·8% Sn)	8% " " *p*H 7·0	"
‖Sodium stannitartrate (17·4% Sn)	8% " " *p*H 7·0	"
§Sodium stannous tartrate (19% Sn)	1% " " *p*H 7·0	"

* Prepared by Professor Van der Kerk, Utrecht, and supplied by the Tin Research Institute.

† Prepared by Dr. W. N. Aldridge.

‡ Prepared by the method of Seifter and Rambousek (1943).

§ Prepared by Dr. J. W. Price, Tin Research Institute.

‖ Prepared by Dr. D. F. Heath.

p.o., by mouth; i.p., intraperitoneal; i.v., intravenous; p.c., percutaneous.

Neuromuscular conduction was studied in rabbits under urethane and in a hen under pentobarbitone anaesthesia. The exposed sciatic nerve was stimulated by supramaximal rectangular impulses of 200 μsec. duration, and the contractions of the gastrocnemius or tibialis anterior were recorded with a Brown-Schuster myograph. The rat phrenic nerve-diaphragm preparation (Bülbring, 1946) was also used, as modified by Barnes and Duff (1953). The experiments on frogs were done during March, and the contractions of the isolated rectus muscle, suspended in frog-Ringer's solution, were recorded with an isotonic lever.

Blood sugar was determined by the method of Haslewood and Stookman, and blood non-protein nitrogen with Nessler's reagent, both as described by King (1951). The blood haemoglobin was determined by Haldane's method. The techniques for the estimation of the muscle phosphates have been described by Stoner, Threlfall, and Green (1952). Cholinesterase activities were determined as described by Davison (1953). Tin was determined polarographically (Godar and Alexander, 1946) using hydroxylamine HCl to reduce ferric iron.

Some of the rats had their adrenals medullectomized by the method of Evans (1936) as described in Farris and Griffith (1942). The completeness of the medullectomy was checked histologically at the end of the experiment. Intravenous injections in unanaesthetized rats were given by the technique of Ginsburg and Heller (1953).

The histology of the tissues was studied on material fixed in 10% (v/v) formol-saline or Helly's fluid. The techniques used for the central nervous system were those of Barnes and Denz (1953).

RESULTS

Acute Toxicity of Alkyl Tin Compounds

Rat.—Triethyl tin sulphate was equally lethal on intravenous, intraperitoneal, and oral administration. Intravenously, rats tolerated 5 mg./kg. body weight, but 10 mg./kg. was fatal in 4 days and higher doses caused immediate death. When groups of 5 rats were given triethyl tin sulphate intraperitoneally in doses of 2, 4, 8 and 16 mg./kg. the mortality after a week was 0, 0, 5, 5, giving an LD50 of 5.7 mg./kg. calculated by the method of Weil (1952). A certainly fatal dose was 10 mg./kg. intraperitoneally which caused death usually within 5 days. Higher doses by this route shortened the survival time, so that 40 mg./kg. was fatal in 2 hr. This compound was less effective when given subcutaneously; when a single dose of 25 mg./kg. was applied, in dimethyl phthalate, to the epilated skin of 4 rats, only 1 died although all became ill.

After the intraperitoneal injection of 10 mg./kg. triethyl tin sulphate striking changes appeared within 30 min. The main effect was a generalized weakness, first manifest in a dragging of the hind limbs. This persisted for 3–4 hr., when the rat recovered slightly. However, by the next day the weakness was more marked and generalized and progressed until the rat died, usually on the 3rd day after the injection. The respiratory rate was slightly depressed and there was vasodilatation in the ears. This latter was very marked after 20 mg./kg. The intensity of these changes was rather less in fed than in fasted animals. Although very ill, the rats continued to attempt to eat and drink until they died. The desire for water seemed increased shortly after the injection, but when weakness became profound the intake of food and water was greatly reduced. An inconstant effect was the secretion of "red tears."

Changing the dose or route of injection did not alter this picture. Larger doses accentuated the weakness so that rats were unable to right themselves 20 min. after the intraperitoneal injection of 30–40 mg./kg. The effects of 10 mg./kg. intraperitoneally could be exaggerated by raising the environmental temperature to 30° C.; the animals quickly became prostrate, and "red tears" were more constant, but the survival time was not shortened.

Trimethyl tin was less toxic. An intraperitoneal dose of 8.8 mg./kg. trimethyl tin sulphate, equivalent in Sn content to 10 mg./kg. triethyl tin sulphate, had no immediate effect. Twenty-four hours later the rats were very excitable, standing up in pairs facing each other as if sparring. This went on almost continuously for a further 24 hr., after which they gradually returned to normal behaviour. Only 1 of a group of 4 died after this dose. With twice this dose of trimethyl tin there was difficulty in moving the hind limbs, and after 24 hr. they exhibited almost continuous, generalized tremors. "Red tears" were seen at this time and death occurred in 2–3 days. The certainly fatal oral dose of this compound was 30 mg./kg.

Various other alkyl tin compounds were examined and their commonly fatal doses, or, for the less toxic members, the highest dose given, are shown in Table II. The occasional difference between the toxicity of oral and intraperitoneal doses suggests that compounds other than the trimethyl and triethyl are poorly absorbed from the gut. There was also a marked difference between the diethyl and triethyl compounds. Although, on intraperitoneal injection, the former was not much less toxic than the latter, the certainly fatal dose being 15 mg./kg., it did not produce any of the characteristic effects of the triethyl tin. After diethyl tin the rats merely became ill, and eventually died without showing

TABLE II

THE CERTAINLY LETHAL DOSES (MG./KG. BODY WEIGHT) OF A RANGE OF ORGANIC TIN COMPOUNDS

Compound	Rabbit		Rat		Guinea-pig
	Oral	i.p.	Oral	i.p.	Oral
Trimethyl	—	—	30	16	—
Triethyl	10	10	10	10 (LD50 5·7)	5–10
Tri-*n*-propyl ..	—	—	>40	—	—
Tri-*iso*-propyl ..	—	—	80	—	—
Tri-*n*-butyl ..	60	—	50–100	10	20
Tri-*n*-hexyl ..	—	—	>100	—	—
Triphenyl	>40	—	>150	—	10
Diethyl phenyl ..	—	—	50–100	—	—
,, dichloride	—	—	>40	15	—
,, diiodide ..	—	—	100	26	—
Dibutyl dichloride	—	—	100	—	—
,, dilaurate ..	—	—	—	85	—
Monoethyl trichloride	—	—	—	200	—

any distinctive changes. The two compounds also differed in their response to dimercaprol (BAL). Premedication and continued therapy with dimercaprol (30 mg./kg. intramuscularly twice a day) had no effect on the response to triethyl tin, but it antagonized the effects of diethyl tin (Table III). When tested in equimolecular amounts the toxicity of diethyl tin dichloride and diethyl tin diiodide was the same. Diethyl tin was equally toxic to the mouse and rat.

Monoethyl tin trichloride, given intraperitoneally (200 mg./kg.), was much less toxic than the other compounds and produced no distinctive symptoms.

TABLE III

EFFECT OF DIMERCAPROL (BAL) (30 MG./KG. I.M. IN OIL) ON THE SURVIVAL TIME OF RATS GIVEN 20 MG./KG. DIETHYL TIN DICHLORIDE (I.P. IN TWEEN 80)

Untreated rats were given equivalent volume of arachis oil i.m. Number of rats shown in parentheses

Treatment	Survival Time	
	Treated	Untreated
BAL given 30 min. before and 6 hr. after injection of diethyl tin	3 days (1) 7 ,, (2) >7 ,, (1)	5½ hr. (1) <24 ,, (3)
BAL given at 2 and 6 hr. after injection of diethyl tin	>7 ,, (3)	<24 ,, (3)

Rabbit.—The rabbit was more sensitive to triethyl tin sulphate than the rat, so although 1.0 mg./kg. intravenously had little effect beyond causing a few running movements at the time of the injection and some vasodilatation in the ears, 3.0 mg./kg. was fatal within 24 hr. After this dose the initial struggling was more severe and respirations were increased in depth. The limbs quickly became flaccid (hind>fore) and there was difficulty in righting for about 15 min. At 5.0 mg./kg. there was a greater initial increase in the rate and depth of respiration, often amounting to stridor, sometimes with squealing and rubbing of the nose. Complete flaccid prostration quickly followed and lasted about an hour. During this period breathing became shallow and rather rapid. The ear vessels were dilated, the pupils were contracted, and nystagmus was sometimes seen. The corneal reflex was sluggish but never absent. After this period of prostration the rabbit usually recovered sufficiently to move about the cage. Some time (1–5 hr.) later, depending on the dose, involuntary tremors appeared and became progressively worse until within 1–2 hr. the rabbit was having continuous violent convulsions. These persisted for an hour or so until the rabbit finally collapsed and died. After 10 mg./kg. intravenously the rabbit usually passed directly from prostration to convulsions and death within 3 hr. Death sometimes occurred while the animal was prostrate. The intraperitoneal or oral administration of 10 mg./kg. triethyl tin sulphate produced the same sequence of events as 5.0 mg./kg. intravenously. Continuous tremors could also be produced by applying it to the skin.

Trimethyl tin was again less effective. An intravenous dose of trimethyl tin sulphate equivalent in Sn content to 5.0 mg./kg. triethyl tin sulphate had no immediate effect other than transitory contraction of the pupil. However, 24 hr. later the rabbit showed the same type of convulsive movements as after triethyl tin although they were less severe. Muscular weakness also occurred after twice this dose of trimethyl tin, but the survival time was longer than after the triethyl compound. The effects of the tributyl and triphenyl compounds are shown in Table II.

After tetraethyl tin (25 mg./kg. i.v.) the only immediate effects were a slight increase in respiratory rate and vasodilatation in the ears. However, 1.5–2.0 hr. later muscular weakness and prostration appeared and then the sequence of events was as after triethyl tin and the mode of death the same.

The intravenous lethal dose of diethyl tin dichloride was 10 mg./kg. The only immediate effect was a short-lived contraction of the pupil and excessive salivation. Death occurred without any of the characteristic signs seen after triethyl tin.

Monoethyl tin trichloride was much less active than the other alkyl tin compounds. Even 150 mg./kg. i.v. was not fatal, but 70–150 mg./kg. by this route produced certain short-lived effects. Almost immediately after the injection there was hyperpnoea, vasodilatation in the ears, some struggling followed by prostration, muscular

tremors and head drop. Within an hour the rabbit had largely recovered and moved about normally.

Domestic Fowl.—In the adult hen 3.0 mg./kg. triethyl tin sulphate intravenously caused immediate collapse with salivation and a few convulsive movements leading to death in a few minutes. After 2.0 mg./kg. there was again immediate prostration with flaccidity of the muscles. The eyes closed and there was excessive salivation. Respiration, after an initial increase in depth, became slow and shallow. The bird was unable to right itself and remained lying on the floor of the cage, apparently unconscious, for 1½–2 hr. It then gradually recovered and on the following day was apparently normal. Prolonged effects were not produced. The birds either died shortly after the injection or recovered completely within a few hours.

Trimethyl tin was about as toxic as the triethyl compound, but the survival time was longer and the immediate effects, though similar, were less severe. After a dose equivalent to 3.0 mg./kg. triethyl tin sulphate the initial effect lasted about ½ hr., after which, although fully conscious, the hen was unable to stand or hold its head up. This state continued until death occurred within 24 hr., preceded by tremors and convulsions as in the rabbit.

In male chicks (about 100 g. body weight) similar responses were observed to these doses of triethyl and trimethyl tin, although there was very little immediate effect after fatal doses of the latter.

Guinea-pig.—Systematic tests were not done on this species, but a sufficient number of oral doses were given to show that, whereas the fatal dose of triethyl tin was of the same order as in the rat and rabbit, the guinea-pig was more sensitive to the oral administration of tributyl and triphenyl tin (Table II). Triethyl tin could also be absorbed through the skin of the guinea-pig.

Chronic Toxicity of Alkyl Tin Compounds

Rat.—On a diet containing 20 parts per million triethyl tin hydroxide the rats ceased to gain weight (Fig. 1). Paired feeding showed that this was owing to decreased food intake. After 7 days, weakness of the hind legs appeared. This progressed until the rat lay on its side on the floor of the cage. It was cold and breathed slowly. The feet were pink, and, although the hind limbs appeared to be "paralysed," they were withdrawn if the foot was pinched. Even in this condition the rats would still attempt to eat, although the intake of food and water was greatly reduced. These changes reached a maximum in 3–4 weeks, when some of the rats died. This picture was not altered by giving additional aneurine hydrochloride (5 μg./100 g. body weight i.p. daily). If they were returned to the normal diet at this stage signs of poisoning disappeared in about 7 days and in 4 weeks they had reached the body weight of the controls (Fig. 1). About half of those kept on the diet containing triethyl tin hydroxide died at this stage, but the remainder appeared to become resistant to the drug, regaining weight and muscular power (Fig. 1). This recovery process was not altered by increasing the concentration of triethyl tin to 40 parts per million, but at double this concentration they lost weight (Fig. 1) and developed generalized muscular tremors like those of acute trimethyl tin poisoning. This particular group were then killed for histological examination, but these tremors were also recorded in another slightly different experiment. Here the rats were fed 20 parts per million of triethyl tin hydroxide for 24 days, to produce severe poisoning, and were then returned to the normal diet. Eight days later, when they had largely recovered, the triethyl tin diet was resumed and severe tremors appeared after a further 7 days. Although the level of the triethyl tin in the diet was maintained, the intensity of the tremors gradually diminished.

Rabbit.—Rabbits tolerated 20 parts per million of triethyl tin hydroxide in the diet, but at 40 and

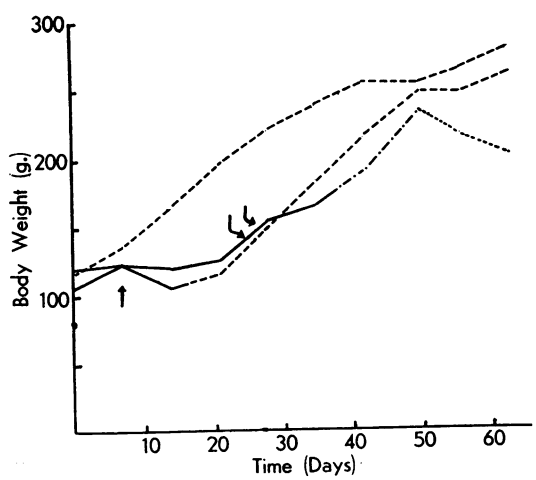

FIG. 1.—The effect of feeding triethyl tin hydroxide on the body weight of rats. Each line shows the mean body weight in a group of 5 rats. First arrow indicates the onset of muscular weakness in the treated groups; other arrows indicate death. - - - - - - - Control diet 41. ——— Control diet 41 + 20 parts per million triethyl tin hydroxide. —.—.—.— Control diet 41 + 40 parts per million triethyl tin hydroxide. ..... Control diet 41 + 80 parts per million triethyl tin hydroxide.

80 parts per million they developed progressive muscular weakness and loss of weight. The hind limbs were affected first and the rabbits sat hunched up in a characteristic posture. The weakness extended to the fore limbs and, in the end, the rabbit lay sprawled out on the floor of the cage. The limbs were markedly wasted. At the 80 parts per million level, muscular weakness could be produced in eight days without any decline in food intake. These diets were ultimately fatal, but there was considerable variation in both onset of signs and survival time. No tremors or convulsions were seen in these rabbits, nor was there any change in haemoglobin concentration.

Domestic Fowl.—Hens tolerated triethyl tin in their diet much better. Even after 15 weeks on a diet containing up to 160 parts per million no ill effects were observed in three hens except for loss of weight due to their dislike of the powdered diet. Because of this the effect of direct oral administration was observed in one hen, and 2.5 mg./kg. triethyl tin hydroxide in arachis oil was given six times a week for four weeks. This had no effect and so the dose was changed to 5.0 mg./kg. triethyl tin sulphate in 0.9% NaCl. After a further month the hen lost weight and developed some muscular weakness.

Toxicity of Other Tin Compounds

Comparison between the behaviour of these alkyl derivatives and that of other tin compounds was difficult. Neutral aqueous solutions of inorganic tin salts cannot be prepared. Stannic chloride in arachis oil was given intraperitoneally to rats without effect, but as a white precipitate was later found in the peritoneal cavity it is doubtful if any was absorbed. Stannic sodium chloride, soluble in aqueous solution at *p*H 3.0, was injected slowly intravenously in rabbits so as to give the amount of tin in effective doses of triethyl tin. The only effect observed was severe hyperpnoea. Of the organic compounds, equivalent doses of stannic lactate were without effect. Stannic citrate was also tried, but, as found by Seifter and Rambousek (1943), it owed its toxicity in acute experiments to its citrate content. More success was obtained with the double salts of tin with sodium tartrate. Three such preparations were used, all proving toxic in the rabbit. Seven daily intravenous doses of 200 mg./kg. of a preparation of sodium stannitartrate containing 33% Sn produced weakness of the hind limbs, loss of weight, and death five days after the last dose. Another preparation containing 17.4% Sn caused death after a single dose of 580 mg./kg. given intravenously. Daily subcutaneous doses, 145 mg./kg., of this compound caused death after 12 days. This compound produced tremors and convulsions similar to those seen after triethyl tin. Sodium stannous tartrate (19% Sn) was also active, and a dose of 132 mg./kg. intravenously, repeated after 24 hr., proved fatal. The effects of all three compounds were delayed in onset, and it was the late effects of triethyl tin which were simulated.

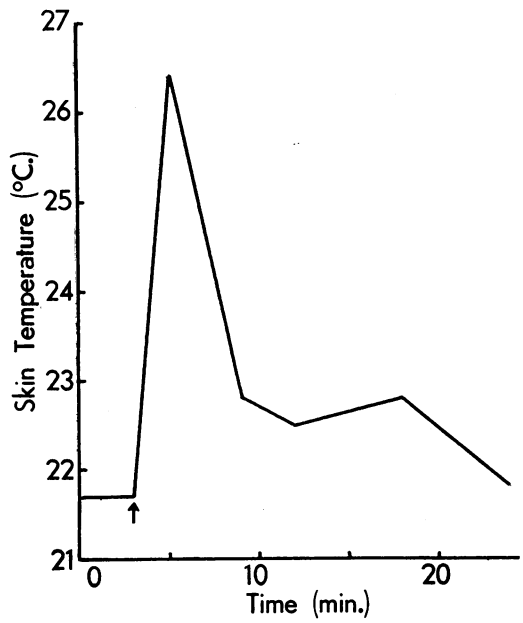

FIG. 2.—The effect, on the skin temperature of the opposite ear, of injecting 10 mg./kg. body wt. triethyl tin sulphate into the ear vein of a rabbit. Time of injection shown by arrow.

Pharmacological Actions of Alkyl Tin Compounds

Cardiovascular System.—Vasodilatation in the ears was noted above. The response in the rabbit, as reflected in the skin temperature of the ear, is shown in Fig. 2. Rectal temperature was depressed in both rat and rabbit. On the day following the intraperitoneal injection of 10 mg./kg. triethyl tin sulphate in the rat this temperature was about 34° C., and fell slowly to 28–30° C., when the animal died. After 20 mg./kg. the rectal temperature fell more rapidly—8–10° C. during the first 5 hr. after the injection. In the rabbit, after 5.0 mg./kg. triethyl tin sulphate, the rectal temperature rapidly fell about 3° C.; it remained at the low level until the tremors appeared, when it rose. The fall in rectal temperature probably resulted from increased heat loss with compensatory vasoconstriction (Grayson,

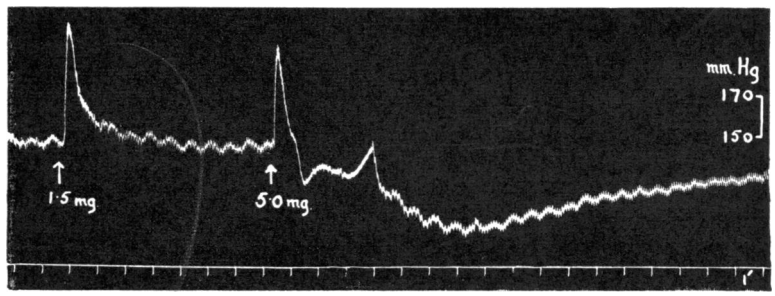

FIG. 3.—The effect of the intravenous injection of triethyl tin sulphate on the blood pressure of a cat (2.2 kg.) under sodium pentobarbitone anaesthesia.

1951). However, recent observations (unpublished) on the effect of triethyl tin on the temperature of the liver and brain suggest that this may not be the whole explanation.

Small intravenous doses (0.12–0.57 mg./kg.) of triethyl tin sulphate raised the blood pressure in the cat. With repeated doses of this order some tachyphylaxis was seen. After larger doses (2.5 mg./kg.) a secondary depressor effect was observed (Fig. 3), becoming more marked as the dose was further increased. Neither the rise nor the fall in blood pressure was significantly altered by atropine sulphate (1.0 mg./kg. i.v.) or splanchnic nerve section. The responses in the rat were rather variable. Most commonly there was a fall in pressure with 0.25 mg./kg. i.v. In the rabbit depressor responses were seen after the intravenous injection of 3.0 mg./kg. triethyl tin sulphate. Trimethyl tin was less active as a depressor agent in this species.

Neuromuscular Conduction.—In the rabbit intravenous doses of triethyl tin sulphate up to 3.0 mg./kg. affected the response of the tibialis anterior and gastrocnemius to fast, but not slow, rates of stimulation through the nerve. The response to 500 stimuli/sec. was first affected so that the muscle was unable to hold the tetanus (Fig. 4). This effect was maximal about 5 min. after the injection, and recovery occurred after about 60 min. even after doses within the lethal range. Responses to rates of stimulation below 200/sec. were not affected by the doses used. These changes could not be correlated with those in the blood pressure, which had returned to the pre-injection level at the time of the maximum effect. Trimethyl tin sulphate had a similar, though less pronounced, action.

In contrast, normal responses were obtained to rates of stimulation up to and including 500/sec. in rabbits fed triethyl tin hydroxide (80 parts per

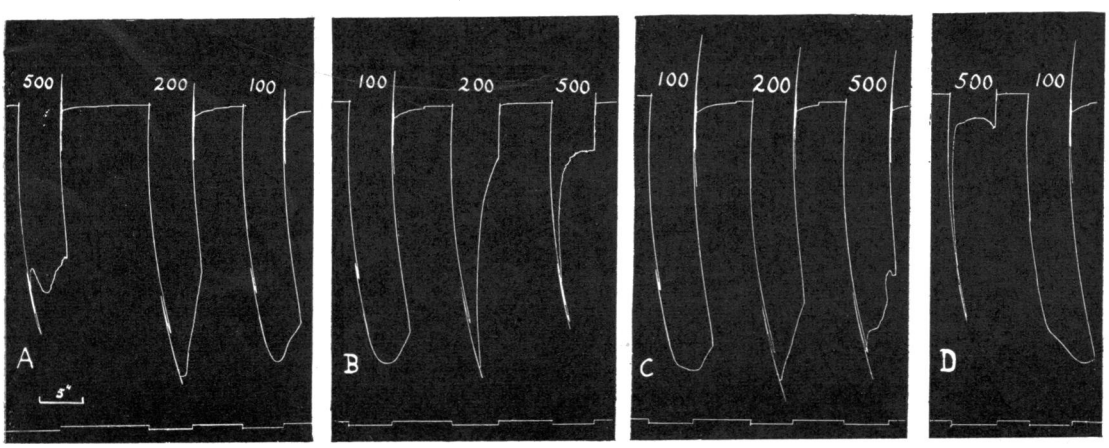

FIG. 4.—The effect of triethyl tin on the response of the tibialis anterior muscle to stimulation of the sciatic nerve in a rabbit (2.5 kg.) under urethane anaesthesia. A, Before administration of triethyl tin; B, 6 min. after 3.0 mg./kg. body wt. triethyl tin sulphate i.v.; C, one hr. later; D, 17 min. after a further dose of 3.0 mg./kg. triethyl tin sulphate. The period of stimulation is shown by the lower tracing; the frequency of stimulation (stim./sec.) is indicated by the numeral above each response.

million) and showing severe weakness of the hind legs. Normal gastrocnemius responses were also found in an isolated experiment on a hen after the intravenous injection of up to 3.0 mg./kg. triethyl tin sulphate.

Rat Phrenic Nerve-Diaphragm.—Triethyl tin (1×10^{-5} M) led to a progressive failure of the diaphragm response to single stimuli. Failure to respond became almost complete within 1 hr. and was often associated with some degree of contracture. On removing the tin some recovery took place. At 5×10^{-5} M complete failure and a marked, irreversible contracture developed rapidly. At 2.5×10^{-6} M there was no change in the response to single stimuli for at least 2 hr., but the preparation by then would no longer hold a tetanus to 500 stimuli/sec. applied for 5 sec. At 1×10^{-5} M there was a failure to hold a tetanus to 50 stimuli/sec. In neither case was recovery complete after removal of the triethyl tin.

With trimethyl tin, on the other hand, the effects were rapidly reversed after removing the tin. A concentration of 1×10^{-4} M was required to cause a failure to hold a tetanus to 50 stimuli/sec., but this concentration had only a slight effect on the size of the response to single stimuli.

The response of the diaphragm to direct stimulation was not affected by triethyl tin (1×10^{-5} M) or trimethyl tin (5×10^{-5} M) until there had been a gross depression of the responses to indirect stimulation. Diethyl tin dichloride (1.5×10^{-4} M) had no effect on the tetanic response to phrenic nerve stimulation, but after 5 min. exposure it produced an irreversible contracture.

Trimethyl and triethyl tin enhanced the action of both (+)-tubocurarine and decamethonium on the diaphragm. In a concentration of 1×10^{-7} M, triethyl tin had no direct action on the preparation, but within 30 min. it led to a complete failure from a dose of tubocurarine or decamethonium that had previously caused only a 50% reduction in the size of the contraction. The effect in neither case was reversed when the tin was removed. Trimethyl tin had a similar effect in a concentration of 5×10^{-6} M, but here the effect was rapidly reversed when the tin was removed. When doses of tubocurarine were added at 10 min. intervals to a medium always containing the tin compounds, the enhancing effect of the tin was immediately detectable, but became progressively greater and did not reach a maximum for at least 30 min. with the lowest effective concentrations used. Higher concentrations of tin caused more rapid changes in the response to tubocurarine or decamethonium.

Frog Rectus Muscle.—On the frog rectus muscle triethyl tin caused a contracture in concentrations above 5×10^{-6} M. Trimethyl tin had a similar action above 5×10^{-4} M. Their action was irreversible and not antagonized by pentamethonium. In doses below those causing direct effects triethyl and trimethyl tin had no effect on the response of the frog rectus muscle to tubocurarine, acetylcholine or decamethonium.

Metabolic Effects of Alkyl Tin Compounds

The methods available for Sn determination were not sufficiently sensitive to enable the distribution of the relatively small toxic doses of tin to be accurately plotted within the tissues. The brain, lungs, liver, kidney, spleen, muscle, heart, pancreas, fat and blood were examined in a number of rats and rabbits after both the oral and intravenous administration of triethyl tin. The tin appeared to be distributed fairly uniformly throughout the tissues. In the rat the concentration in the blood (20–30 μg./ml.) was maintained during the 3-day period between the injection of the tin and the death of the animal. Over this period no concentration of the tin within any single organ or tissue was detected.

When the higher alkyl and phenyl derivatives were given to rats by mouth in oil, a considerable proportion was excreted in the faeces, and this may partly account for their lower toxicity.

TABLE IV

THE EFFECT OF TRIETHYL TIN SULPHATE (10 MG./KG. I.P.; *p*H 7.4; 37° C.) ON THE BLOOD-SUGAR CONCENTRATION IN NORMAL RATS, AND IN RATS 67 DAYS AFTER ADRENAL MEDULLECTOMY

Each value is the mean from 2–5 animals. The rats were starved overnight before the expt., the injection was given at 10 a.m., after which they had access to food. Control animals were given an equivalent volume of 0.9% NaCl

Time after Injection (hr.)	Blood Sugar Concn. (mg./100 ml. Blood)		
	Normal Controls	Norm	Medullectomized
0	74	—	66
0·5	67	121	51
1·0	55	101	—
2·0	56	109	—
4·0	61	117	73
6·0	61	119	—
24	115	123	—
48	129	105	—

A moderate hyperglycaemia followed the intraperitoneal injection of triethyl tin sulphate, but not after adrenal medullectomy (Table IV). The non-protein nitrogen level of the blood was increased to between 68 and 92 mg./100 ml. blood 24 hr. after the intraperitoneal injection of 10 mg./kg. triethyl tin sulphate, and continued at that level until the rat died.

In vitro, triethyl tin (1.4×10^{-3} M) caused a 25% decrease in the pseudo-cholinesterase activity of horse serum. In the rat the true and pseudo-cholinesterase activities of the brain and spinal cord were unchanged 4 hr. after the intraperitoneal injection of 10 mg./kg. triethyl tin sulphate. Observation on rats fed triethyl tin hydroxide (20–80 parts per million) showed that severe signs of poisoning could be produced without significant alteration in the true or pseudo-cholinesterase activity of the spinal cord.

The affected hind limb muscles were also examined. It was found that the total acid soluble phosphate, inorganic phosphate, phosphocreatine and nucleotide (7 min. acid hydrolysable) phosphate fractions were unaltered 30 min. and 4 hr. after the injection of 20 mg./kg. and 52 hr. after the injection of 10 mg./kg. triethyl tin sulphate intraperitoneally in rats.

Morbid Anatomy of Alkyl Tin Poisoning

Autopsies on animals given alkyl tin compounds revealed little to the naked eye. After intraperitoneal injection there was a slight increase in the fluid of the peritoneal cavity with occasional small pleural effusions. These latter were more constant and larger after the sodium tin tartrates. A further change after these compounds was in the appearance of the spleen. Its size was within the normal range, but it was pale, with a bluish white tinge ; in one case it was almost white. The concentration of Sn in the tissues of one rabbit which had received a total of 1.16 g./kg. sodium stannitartrate was estimated, and, although only about half the injected tin could be accounted for in the tissues, the concentration in the spleen was about 10 times greater than that in any of the other tissues. These changes in the spleen suggest that, although neutral solutions of the sodium tin tartrates can be prepared, alterations occur soon after injection whereby the double salts are removed from the blood stream by phagocytosis.

Routine histological examination of the main organs in animals given triethyl tin failed to reveal any abnormality. A more detailed study of the central nervous system in these animals is in progress. In the animals fed triethyl tin in the chronic toxicity experiments extensive damage was found in the central nervous system, and an account of this will be given elsewhere (Magee, Stoner, and Barnes, to be published).

Discussion

Previous work on the alkyl tin compounds, except for that of McCombie and Saunders (1947), has been confined to the triethyl and tetramethyl derivatives. McCombie and Saunders (1947) examined a range of these compounds, but were solely concerned with their sternutatory action. This is present, but not marked, in the members of the series studied here. The only toxicological studies are those of White (1881) and Ungar and Bödlander (1886), who examined the effect of triethyl tin acetate in frogs, rabbits, dogs and cats. Similar results with tetramethyl tin were reported in a note by Seifter (1939a). The present results confirm much of this earlier work. In addition to giving information of their activity in additional species—the rat, guinea-pig, and fowl—it is now possible to consider the comparative toxicity of a series of alkyl tin compounds.

The most active compounds were the tri- and di-alkyl tin compounds. Tetraethyl tin was less active than triethyl tin, but the similarity of its effects after a latent period suggests the possible *in vivo* conversion of the tetra- to the tri-alkyl derivative. The most active members of both the tri- and di-substituted series were the ethyl compounds ; but this is their only similarity, for not only are their toxicological effects different but the antagonistic action of dimercaprol on diethyl tin suggests that it acts through combination with -SH groups. The establishment of diethyl tin as a -SH inhibitor must await further study, as must the position of the mono-alkyl derivatives.

Triethyl tin was the most active of the tri-alkyl compounds, and the central nervous system was the site of the main action. The typical response seems to be that in the rabbit where, after the intravenous injection of triethyl tin, the phases of initial prostration, partial recovery, and final encephalopathy are clearly seen. By suitable modification of dose, route, and member of the series used, it can be reproduced in the rat and fowl. White (1881) and Ungar and Bödlander (1886) had previously shown its occurrence in the dog and cat. Although much of the earlier work was confirmed, diarrhoea was less often found except as a terminal event after the oral administration of the poorly absorbed compounds. The toxicity of the double salt of tin with sodium tartrate was finally confirmed, although the doses required were larger than those used by White (1881) and Ungar and Bödlander (1886). It is difficult to determine from their papers which of the three possible salts they used. In acute experiments the most active compound, sodium stannous tartrate, was apparently twenty times less toxic than triethyl tin, on the basis of Sn content. This may, however, be a serious overestimate of the difference, since these

double salts may, after injection, form particles which are removed from the circulation by the spleen, so reducing the effective concentration. More important than the difference in toxicity level is the fact that these double salts reproduced the delayed effects of trialkyl tin poisoning. The immediate "narcotic" action was peculiar to trialkyl tin.

The mode of action of the trialkyl tin compounds is not known nor, at the moment, can we advance a single hypothesis satisfactory for all the compounds examined. The earlier workers had concluded that the main effect was on the central nervous system, which is certainly the most obvious site of action. Although the tin is not concentrated in the brain or spinal cord the various peripheral effects seem insufficient to account for the general effect. Work on the action of alkyl tin compounds on the metabolism of tissues *in vitro* is in progress, and although no unifying biochemical lesion can yet be postulated it would seem reasonable to suppose that such a lesion has occurred. The absence of gross pathological lesions in any important organ, despite an interval of several days between the administration of a lethal dose of tin and the death of the animal, supports such a concept; nor is the argument disturbed by the demonstration of widespread damage in the central nervous system of *chronically* poisoned rats, for the diffuse nature of the damage suggests that it is the result of a biochemical lesion. The signs of poisoning suggest that whatever part of the metabolic cycle is disturbed by tin this is more vital to the proper functioning of the brain than to that of other organs.

The peripheral actions themselves give little help in the isolation of the lesion. In some respects these actions resemble those of onium ions, to which chemical group these compounds might be considered to belong. However, the dissociation constant of triethyl tin hydroxide is 3.35×10^{-8}, indicating that only very weak onium ion activity could be expected (Ing, 1936). The action of these compounds on isolated muscle preparations alone and in the presence of (+)-tubocurarine and decamethonium creates further difficulties in their classification into any well-defined pharmacological group. Since they are not without action on the muscle itself it is not even possible to say that their action is limited to the myoneural junction, although they may act there preferentially.

The decreasing toxicity on either side of the triethyl compound is partly a matter of absorption. The striking difference between the compounds when given by mouth is not seen in the guinea-pig, where absorption from the gut appears more efficient, nor is it seen when they are given parenterally.

The reproduction of the late effects of the trialkyl tin compounds by large doses of sodium tin tartrates is the nearest that has been reached to a comparison with inorganic tin compounds. The insolubility of the latter, and of most other tin compounds, in biological fluids is the main reason for their pharmacological inertness. The pharmacological relationship between the trialkyl tin compounds and other forms of tin appears similar to that between such metals as lead, antimony, bismuth and mercury and their trialkyl derivatives (Harnack, 1878; Seifter, 1939b; Sollman and Seifter, 1939; Hunter, Bomford, and Russell, 1940). In all of these the presence of the alkyl groups enhances the toxicity of the metals —apparently by aiding the absorption of the metal and its distribution to the site of action. Whether the whole difference between the toxicity of the metal and its alkyl derivatives and the immediate effects of the latter can be explained thus is not known. From a practical point of view the striking central nervous system actions of the alkyl tin compounds require that they should be handled with care, particularly during manufacture.

Summary

1. The biological effects of a series of tetra-, tri-, di- and mono- alkyl tin compounds, and of some other organic tin compounds, have been studied in rats, rabbits, guinea-pigs and fowls, in both acute and chronic experiments.

2. The di- and tri- ethyl tin compounds behave differently and only the toxic effects of the former are antagonized by dimercaprol.

3. In acute experiments on rabbits, triethyl tin, the most active compound, produces muscular weakness followed by some recovery, followed in turn by muscular tremors proceeding to convulsions and death. The other species and compounds show variations of this pattern.

4. The outstanding sign of chronic poisoning is muscular weakness.

5. Although there is no evidence that the tin is concentrated in any particular organ the main site of action of these alkyl compounds appears to be in the central nervous system.

6. Since the delayed effects of triethyl tin poisoning are reproduced by large doses of sodium tin tartrate it is thought that the pharmacological relationship between the triethyl tin compounds and other forms of tin is similar to that between other metals (Pb, Hg, Sb) and their alkyl derivatives.

7. Because of their marked effect on the central nervous system caution is advised in the handling of these compounds.

We wish to thank Mr. A. N. Davison for the cholinesterase determinations, Dr. W. N. Aldridge for the measurement of the dissociation constant of triethyl tin hydroxide, and Dr. P. N. Magee for help with the histology. Our thanks are also due to Miss P. Harrison and Messrs. J. A. E. Jarvis and K. Lorenzen for their technical assistance.

Mr. W. R. Lewis and Dr. J. W. Price, of the Tin Research Institute, supplied us with most of the compounds used in this work, and we are grateful to them for their co-operation.

ADDENDUM

Since this paper was submitted for publication it has come to our notice that there have recently been 110 deaths in France in cases of furunculosis treated orally with a proprietary preparation containing diethyl tin diiodide dissolved in linoleic acid. The total amount of diethyl tin diiodide consumed by these patients appears to have been about 3.0 g.

Our thanks are due to M. Vaille, of the French Ministry of Health, and Dr. Le Breton, of the Institut Medico-Legal, Paris, for information about patients receiving diethyl tin.

REFERENCES

Barnes, J. M., and Denz, F. A. (1953). *J. Path. Bact.*, **65**, 597.

—— and Duff, J. I. (1953). *Brit. J. Pharmacol.*, **8**, 334.

Bruce, H. M. (1947). *J. Hyg.*, **45**, 169.

—— and Parkes, A. S. (1949). Ibid., **47**, 202.

Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38.

Davison, A. N. (1953). *Biochem. J.*, **54**, 583.

Eckhardt, A. (1909). *Z. Untersuch. Nahr.-u. Genussm.*, **18**, 193.

Evans, G. (1936). *Amer. J. Physiol.*, **114**, 653.

Farris, E. J., and Griffith, J. Q. (1942). *The Rat in Laboratory Investigation.* London: Lippincott.

Ginsburg, M., and Heller, H. (1953). *J. Endocrinol.*, **9**, 267.

Godar, E. M., and Alexander, O. R. (1946). *Anal. Chem.*, **18**, 681.

Grayson, J. (1951). *Brit. med. J.*, **2**, 1379.

Handovsky, H. (1925). *Arch. exp. Path. Pharm.*, **114**, 39.

Harnack, E. (1878). Ibid., **9**, 152.

Hunter, D., Bomford, R. R., and Russell, D. S. (1940). *Quart. J. Med.*, **9**, 193.

Ing, H. R. (1936). *Physiol. Rev.*, **16**, 527.

King, E. J. (1951). *Micro-analysis in Medical Biochemistry.* 2nd ed., pp. 11, 23. London: Churchill.

Lehmann, K. B. (1902). *Arch. f. Hyg.*, **45**, 88.

McCombie, H., and Saunders, B. C. (1947). *Nature, Lond.*, **159**, 491.

Salant, W., Rieger, J. B., and Trenthardt, E. L. P. (1914). *J. biol. Chem.*, **17**, 265.

Schryver, S. B. (1909). *J. Hyg.*, **9**, 253.

Schwartzer, E. W., and Clarke, W. F. (1927). *J. Pharmacol.*, **31**, 224.

Seifter, J. (1939a). Ibid., **66**, 32.

—— (1939b). Ibid., **66**, 366.

—— and Rambousek, E. S. (1943). *J. Lab. clin. Med.*, **28**, 1344.

Sollman, T., and Seifter, J. (1939). *J. Pharmacol.*, **67**, 17.

Stoner, H. B., Threlfall, C. J., and Green, H. N. (1952). *Brit. J. exp. Path.*, **33**, 398.

Ungar, E., and Bödlander, G. (1886). *Z. Hyg. InfektKr.*, **2**, 241.

Van der Kerk, G. J. M., and Luijten, J. G. A. (1954). *J. appl. Chem.*, **4**, 314.

Weil, C. S. (1952). *Biometrics*, **8**, 249.

White, T. P. (1881). *Arch. exp. Path. Pharmak.*, **13**, 53.