Biological Activity of Organotin Compounds—An Overview

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As a consequence of the rapid expansion of the uses and applications of the organotin compounds, the concern about their environmental and health effects is increasing. The main subject of this overview is the current understanding of the mammalian toxicity of the organotin compounds. Four different types of target organ toxicity, namely neurotoxicity, hepatotoxicity, immunotoxicity, and cutaneous toxicity, are discussed in more detail. The effects of the organotin compounds on the mitochondrial and cellular level are summarized and discussed in relation to the mode of action of these compounds on the central nervous system, the liver and bile duct, the immune system, and the skin. © 1987 Academic Press, Inc.

ORGANOTIN COMPOUNDS: APPLICATIONS AND TOXICITY

Organotin Compounds

Tin can be present as an element in a wide variety of both inorganic and organometallic compounds. Organometallic tin compounds or organotins are characterized by the presence of at least one covalent carbon–tin bond. Although tin may exist either in the Sn^{2+} or in the Sn^{4+} oxidation state, almost all organotins have a tetravalent structure. Depending on the number of organic moieties, the organotin compounds are classified as mono-, di-, tri-, and tetraorganotins. In compounds of industrial importance, methyl, butyl, octyl, and phenyl groups form the organic substituents, while the anion is usually chloride, fluoride, oxide, hydroxide, carboxylate, or thiolate. All alkyltin compounds refered to in this article contain unbranched saturated hydrocarbon side chains (*n*-alkyltins).

Applications of Organotin Compounds

It was not until 80 years after the synthesis of one of the first organotin compounds by Löwig in 1852 that the organotins found application as stabilizers of transformer oils (patented in 1932) and vinyl plastics (patented in 1940 and 1943). Due to the systematic investigations in the 1950s, especially by Van der Kerk and co-workers (1954, 1958), the commercial uses of the organotin compounds expanded rapidly during the last 40 years. At present there are three major areas of utilization of the organotin compounds, namely as heat stabilizers for polyvinyl chloride polymers, as industrial and agricultural biocides, and as industrial catalysts in a variety of chemical reactions (Lewis and Hedges, 1957; Ross, 1965; Luijten, 1971; Van der Kerk, 1978; Wilkinson, 1984).

Diorganotin compounds and a few monobutyltins are used for heat and light stabilization of vinyl chloride polymers. In particular, dimethyltin, dibutyltin, and

dioctyltin compounds are of economic importance in various applications of PVC-plastics.

The biocidal activity of the triorganotin compounds was first recognized by Van der Kerk and Luijten (1954) and studied later by Kaars Sijpesteyn et al. (1962). Tripropyltin, tributyltin, and triphenyltin compounds especially demonstrated high fungicidal and bactericidal properties. Tributyltin oxide (TBTO) is now used in wood preservation, marine antifouling, disinfection of circulating industrial cooling waters, and slime control in paper mills. The activity of tributyltin and triphenyltin compounds against molluscs has been suggested to be of value in combating the parasite that causes schistosomiasis in man. This debilitating disease can be fought by killing the snails that serve as an intermediate host for the parasitic worms (Cardarelli, 1976; Duncan, 1980). Triphenyltin compounds have become important agricultural fungicides due to their specific activity against two major plant diseases, the late blight on potatoes and the leaf spot in sugar beets. Tricyclohexyltin and trineophyltin compounds are marketed for their activity against phytophagous mites and ticks, which threaten fruit culture.

Organotin compounds are applied as catalysts in the production of polyurethane foams and the curing of silicone rubbers and epoxy resins. Methyltin compounds are used to obtain tin oxide coatings on glass surfaces, for a better resistance against abrasion and chemical corrosion (Van der Kerk, 1978).

Growing Production and Concern

Due to the expansion of technical applications, the annual world production of organotin compounds has grown rapidly from 500 tons in 1950 to 25,000 tons in 1975 (Van der Kerk, 1978), and was expected to grow over 50,000 tons in 1986 (WHO, 1980). Along with this rapid growth, the concern about possible environmental and health effects increased. The anxiety for the toxicity of the organotin compounds was roused particularly by a tragic incident with a proprietary preparation, Stalinon, that was sold in France for the treatment of staphylococcal skin infections. It was said to contain diethyltin diiodide, but most likely due to contamination with triethyltin iodide, 217 people were poisoned in 1954. At least 100 of them died (Alajouanine et al., 1958; Barnes and Stoner, 1959). Since that time, the toxicology of the organotin compounds has received detailed attention (Stoner et al., 1955; Barnes and Stoner, 1958, 1959; Barnes and Magee, 1958; Magee et al., 1957; Stoner, 1966).

In the last decade, the possible environmental hazard of the organotin compounds has become a subject of concern. The increasing use of TBTO as an antifouling agent and the possible large-scale application of triorganotin compounds as molluscicides have necessitated investigations on the occurrence of organotins in the aquatic environment and their toxicity to aquatic organisms (Sheldon, 1975; Zuckerman et al., 1978; Laughlin and Lindén, 1985). From the many studies carried out recently, it is clear that zooplankton species (e.g., copepods; U'ren, 1983), younger developmental stages of macroinvertebrates (e.g., larvae of amphipods and mussels; Laughlin et al., 1984; Beaumont and Budd, 1984), and early life stages of fish (Seinen et al., 1981) are very susceptible to tributyltin exposure. Acute mortality among these species was noticed at tribu-

tyltin concentrations ranging from 0.1 to 5 µg/liter. Adult fish (Chliamovitch and Kuhn, 1977) and bivalves (Thain, 1983) are relatively more resistant to the acute effects of these organotin compounds. Toxicity data for phytoplankton, zooplankton, macroinvertebrates, fish, and amphibians were recently summarized by Hall and Pinkney (1985).

Concomitant with the increased attention for the aquatic toxicity of the organotin compounds, the presence of butyltin and methyltin compounds in natural waters has been investigated. Recently, organotin concentrations with varying intensity were determined in the United States (Hodge *et al.*, 1979; Seligman *et al.*, 1986), Canada (Maguire *et al.*, 1982, 1986), France (Alzieu and Heral, 1984), Turkey (Tugrul *et al.*, 1983), Switzerland (Mueller, 1984), and the United Kingdom (Cleary and Stebbing, 1985). In general, butyltin concentrations seemed to depend on boating activity and tidal influence. Low or undetectable concentrations were found at marine sites, while in harbor waters with high pleasure craft activity organotin concentrations were relatively high. Especially in the surface microlayer, dibutyltin and tributyltin were concentrated, occasionally by a factor of 10^4 relative to the subsurface water resulting in concentrations as high as 2600 and 60 µg/liter, respectively. Therefore, at various sampling sites, the reported tributyltin levels approached or exceeded concentrations demonstrated to be lethal for sensitive aquatic species (Maguire *et al.*, 1982, 1986; Stebbing, 1985). In France and England, a falling in the oyster fisheries was noticed, which was correlated with the occurrence of tributyltin compounds in coastal waters. For this reason, the use of triorganotin-containing paints on vessels less than 25 meters was banned in France in 1982. Although this ban has been incomplete, shell malformations and reproduction problems among the oysters have declined significantly (Alzieu and Heral, 1984; Stebbing, 1985). Recently, the British Department of the Environment has announced measures to reduce the negative impact of organotin-containing antifouling paints on the aquatic environment (Thain and Waldock, 1986).

In 1980, the World Health Organization published various suggestions for further research on the biological effects of organotin compounds (WHO, 1980). In view of the possible application of TBTO for the control of the schistosoma parasite, more informaton was needed for a proper human risk assessment. Therefore, on behalf of the WHO, the immunotoxic, carcinogenic, and teratogenic potential of TBTO is being investigated at present (Krajnc *et al.*, 1984; Vos *et al.*, 1984a; Krowke *et al.*, 1986).

MAMMALIAN TOXICITY OF ORGANOTIN COMPOUNDS

General Aspects

The toxicity of organotin compounds is essentially determined by the number and nature of the organic substituents. In general, the toxicity to mammals decreases from tri- to monoorganotins. The tetraorganotin compounds resemble the triorganotins in their toxicity, but their effects are often less and delayed. This has been explained by a conversion of tetra- into triorganotin compounds in the liver (Cremer, 1958) or in the mucosa of the intestinal tract (Iwai and Wada, 1981). The

toxicity within each class of organotin compounds is determined by the number of carbon atoms per side chain. Within the series of trialkyltin compounds, the lower homologs, trimethyltin and triethyltin, demonstrate the highest toxicity. Further increase in the n-alkyl chain reduces the mammalian toxicity. Trioctyltin compounds are essentially nontoxic. Variation of the anionic radical has, in general, very little effect on the biological activity. The mammalian toxicity of the organotin compounds was summarized by Duncan (1980) and in a WHO report (1980). A list of acute LD_{50} values was given by Smith (1978). Some of them are listed in Table 1. The specific action of the organotin compounds on target organs and organ systems, such as the central nervous system (CNS), the liver and bile duct, the immune system, and the skin will be discussed in more detail.

Neurotoxicity

The toxic action of triethyltin on the CNS of rodents was first described by Stoner *et al.* in 1955. In more detailed studies using adult rats, Magee *et al.* (1957) reported that dietary concentrations of 20 mg triethyltin/kg feed induced interstitial edema of the white matter of the brain and spinal cord without obvious neuronal damage. Edema consisted of a progressive increase in water, sodium, and chloride contents of the CNS and resulted in an increased cerebrospinal fluid pressure (Magee *et al.*, 1957; Leow *et al.*, 1979). Vascular permeability to molecules larger than 3000 D was not altered significantly (Hultström *et al.*, 1984). From various studies, it was concluded that the basic pathologic lesion was limited to myelin. In some rat strains myelin degeneration was noticed upon chronic treatment of adult rats (Smith, 1973). Neonatal exposure to triethyltin caused a reduction of brain weight and a disruption of myelinogenesis, but cerebral edema was not observed (Wender *et al.*, 1974; Reiter *et al.*, 1981; O'Callaghan *et al.*, 1983). To a lesser degree, changes in the peripheral nervous system have also been observed (Gerren *et al.*, 1976; Graham *et al.*, 1976b). Triethyltin has been shown to reduce levels of various neurotransmitters, such as norepinephrine, serotonin, and dopamine in adult rat brain (Moore and Brody, 1961; Bentue-Ferrer *et al.*, 1985). As a consequence of these lesions, electrophysiological alterations

TABLE 1

ACUTE ORAL LD_{50} VALUES FOR VARIOUS ORGANOTIN COMPOUNDS IN THE RAT[a]

Compound	LD_{50} (mg/kg)
Mono-n-butyltin trichloride	2200; 2300
Di-n-butyltin dichloride	219; 126; 112–182
Tri-n-butyltin chloride	122; 349; 129
Tetra-n-butyltin	>4000
Trimethyltin acetate	9
Triethyltin acetate	4
Tri-n-propyltin acetate	118
Tri-n-butyltin acetate	380; 125–136
Tri-n-octyltin acetate	>1000

[a] Values obtained from Barnes and Stoner (1958) and Smith (1978).

(Gerren et al., 1976; Dyer et al., 1981) and behavioral deviations (Squibb et al., 1980; Harry and Tilson, 1981) have been demonstrated in triethyltin-exposed rodents. In adult rats, triethyltin caused hypoactivity and decreased motor function, whereas rats neonatally exposed to triethyltin became hyperactive in later life (Reiter et al., 1981; McMillan and Wenger, 1985). Muscular weakness, at least in part of neuropathologic origin and often resulting in partial or total paralysis, was also frequently observed in chronically intoxicated animals (Graham et al., 1976a; Bierkamper and Bassett, 1984). In man, accidentally exposed to triethyltin in the Stalinon affair, cerebral edema was also the most pronounced finding. Muscular weakness and paralysis were as well observed (Alajouanine et al., 1958).

Although neurologic effects of trimethyltin in rats, such as tremor, hyperexcitability, and aggression, were reported in the original studies of Stoner et al. (1955) and Barnes and Stoner (1958), no histopathologic lesions were described until more than 20 years later. Brown et al. (1979) and Bouldin et al. (1981) observed necrosis of neurons in specific areas of the CNS in rats given several oral doses of 4 or 5 mg trimethyltin/kg body weight. Particularly the hippocampus and the pyriform cortex were affected and in contrast to triethyltin no cerebral edema was found. In subsequent studies, localized neuronal damage was also observed in the amygdaloid nucleus, brainstem, neocortex, spinal cord, sensory neurons, retina, and cochlea (Chang et al., 1982a, b, 1983a, 1984; Chang and Dyer, 1983; Bouldin et al., 1984; Fechter et al., 1986). Although the exact distribution of neuronal alterations and its severity appeared species and strain dependent (Chang et al., 1983b), the special vulnerability of the hippocampal formation was recognized in all studies. From several acute and chronic intoxication studies, it became evident that trimethyltin alters many neurochemical parameters in various brain areas. The concentration of specific neuronal phosphoproteins, such as synapsin I, was decreased (O'Callaghan and Miller, 1984; Harry et al., 1985). Neurotransmitter levels of the GABA-ergic, glutamergic, dopaminergic, and serotonergic systems were changed (Doctor et al., 1982; Valdes et al., 1983; DeHaven et al., 1984; Hanin et al., 1984; Wilson et al., 1986). Concentrations of norepinephrine and acetylcholine remained generally unaltered (Valdes et al., 1983; Hanin et al., 1984). The influence of trimethyltin on rodent behavior has received much attention, and was recently summarized by McMillan and Wenger (1985). In monkeys intoxicated with trimethyltin, similar behavioral and neuropathologic alterations were noticed as in rodents (Brown et al., 1984; Reuhl et al., 1985). Also for humans accidentally exposed to trimethyltin a variety of psychomotor changes were reported, including irritability, depression, aggressiveness, headaches, tremors, convulsions, and changes in libido (Fortemps et al., 1978; Ross et al., 1981).

For all organotin compounds, neurotoxic effects were limited to trimethyltin and triethyltin. No signs of neuronal damage or edema were observed in rats treated with dimethyltin, diethyltin, or any of the higher trialkyltin homologs (Bouldin et al., 1981; Mushak et al., 1982; Snoeij et al., 1985).

Hepatotoxicity

Some monobutyltin compounds were shown to cause steatosis of hepatocytes

and enlargement of the liver when given to mice as a single oral dose of 4 g/kg body weight (Pelikan and Cerny, 1970). Dibutyltin compounds were considerably more toxic to rodents and caused a specific lesion in bile ducts of rats and mice after a single oral dose of 50 mg/kg or more (Barnes and Magee, 1958). This lesion consisted of an inflammatory reaction in the wall of the bile duct, beginning at the lower part near the duodenum. Upon repeated dosing, inflammation of the prox- imal part and proliferation of intrahepatic bile ducts were observed as well. In excessive cases, peritonitis and pancreatitis were found due to perforation of the lining of the bile duct. Since cholangitis was not demonstrated in cats, rabbits, and guinea pigs, this lesion was suggested to occur only in animal species where the bile duct and the pancreatic duct have a common course (Barnes and Magee, 1958). Of the dialkyltin compounds, bile duct damage is primarily produced by dibutyltin but to a lesser degree also by diethyltin, dipropyltin, dipentyltin, and dihexyltin compounds. The occurrence of this lesion was found to correlate with the presence of tin in the bile. Biliary tin concentrations were high in rats treated with dibutyltin, while lower levels were found in rats exposed to dipentyltin and dihexyltin. Almost no tin was recovered from the bile of rats given dioctyltin (Barnes and Magee, 1958; Merkord et al., 1982). The bile duct lesion was also observed in rats chronically exposed to tributyltin compounds (Barnes and Stoner, 1958; Mushak et al., 1982; Krajnc et al., 1984). Moreover, intrahepatic and extrahepatic cholangitis was noticed in rats given a single oral dose of 25 mg tricyclohexyltin/kg body weight (Kimbrough, 1976).

Immunotoxicity

For some of a series of dialkyltin compounds, a selective action on the immune system of the rat was observed (Seinen and Willems, 1976; Seinen et al., 1977a; Miller et al., 1983). Particularly, dipropyltin, dibutyltin, and dioctyltin com- pounds caused a dose-related decrease in the weights of thymus, spleen, and lymph nodes in rats fed these compounds for several weeks. Reduction of thymus weight was already observed at dietary concentrations as low as 5 mg dibutyltin or dioctyltin/kg feed (Seinen, 1980). Histologically, the decrease in lymphoid organ weights was associated with a depletion of lymphocytes in the thymus and thymus-dependent areas of spleen (periarteriolar lymphocyte sheaths) and lymph nodes (paracortical areas). In particular, the thymic cortex was depleted of lym- phocytes, without signs of overt cell destruction (Seinen and Willems, 1976; Pen- ninks et al., 1985). As a consequence, the immune response of rats fed dibutyltin or dioctyltin compounds was affected. Various immune function studies such as the delayed type hypersensitivity to tuberculin, allograft rejection, graft-versus- host reaction and resistance to *Listeria monocytogenes* infection indicated that the cell-mediated immune responses were suppressed by these dialkyltin com- pounds. Also the T-cell-dependent humoral immunity, as reflected by antibody synthesis to sheep red blood cells, was decreased upon dialkyltin exposure. No effect was observed, however, on the thymus-independent antibody production to *Escherichia coli* lipopolysaccharide (Seinen et al., 1977b; Seinen and Pen- ninks, 1979). During the developmental phase of the immune system, the dial- kyltin-induced lymphoid atrophy appeared most severe in rats (Seinen, 1980).

The lower dialkyltin homologs did not (dimethyltin) or only slightly (diethyltin) reduce thymus weights, while homologs with 12 or 18 carbon atoms per side chain (didodecyltin, dioctadecyltin) were again ineffective in reducing thymus weights.

Immunotoxic properties were described for some of the triorganotin compounds as well. Thymus weight reduction, associated with a depletion of cortical lymphocytes, was found in rats fed tripropyltin, tributyltin, and triphenyltin compounds at dietary levels as low as 15 to 25 mg/kg feed (Vos *et al.*, 1984b; Krajnc *et al.*, 1984; Snoeij *et al.*, 1985). Moreover, upon feeding tributyltin or triphenyltin compounds to rats (Snoeij *et al.*, 1985), mice (Ishaaya *et al.*, 1976) or guinea pigs (Verschuuren *et al.*, 1966, 1970), a reduction of spleen weight and a decrease in the number of circulating lymphocytes were noticed. Immune function studies with rats fed tributyltin or triphenyltin compounds revealed that the thymus-dependent immune responses were suppressed. Triphenyltin specifically affected delayed-type hypersensitivity reactions, while tributyltin exerted a wider spectrum of effects. Next to a disturbance of the T-cell-mediated immunity, also parameters of the nonspecific resistance were depressed in TBTO-exposed rats (Vos *et al.*, 1984a, b). For the higher trialkyltin homologs only a limited (trihexyltin) or no effect (trioctyltin) on the thymus was observed. Possible immunotoxic properties of trimethyltin or triethyltin compounds may be overshadowed by their neurotoxicity (Snoeij *et al.*, 1985).

In contrast to tributyltin and dibutyltin, a single oral dose of monobutyltin, given at levels up to 180 mg/kg body weight, did not induce thymus atrophy in rats (Snoeij, 1987).

Cutaneous Toxicity

Various di- and triorganotin compounds exert irritating effects on skin or eyes of rodents and man. Of the dialkyltin compounds, the lower homologs caused skin lesions upon successive daily applications of 80 mg/kg body weight. The most water-soluble compound, dimethyltin, caused necrosis of superficial layers of rat skin, while the more lipophilic homologs, diethyltin and dipropyltin compounds, induced deep-seated inflammation and edema. Dialkyltins with 5 carbon atoms or more per side chain did not affect rat skin. In contrast to rats and rabbits, guinea pigs were relatively resistant to the cutaneous application of dibutyltin dichloride (Barnes and Stoner, 1958). After a single application, the epidermal damage caused by tributyltin was more severe than that by dibutyltin. A dose of 167 nmol dibutyltin/cm^2 did not cause histologic damage, whereas an equimolar dose of tributyltin induced almost total epidermal necrosis and marked dermal inflammation in the skin of rats (Middleton and Pratt, 1978). Triphenyltin, at dose levels up to 90 mg/kg body weight, did not appear to have any action on the skin of guinea pigs (Stoner, 1966). Topical application of 1 g of triphenyltin/kg body weight on the skin of rabbits was without toxic effects, but an amount of 10 mg caused marked irritation of the eye, resulting in corneal opacity (Winek *et al.*, 1978). Tricyclohexyltin was described to be irritant to both skin and eyes of rabbits (Kimbrough, 1976).

Lyle (1958) reported on the irritant effects of butyltin compounds on human skin. When dibutyltin or tributyltin compounds were, accidentally, allowed to lie on the skin for a few minutes, a typical acute organotin burn developed. From a study with volunteers, it was concluded that of a series of dibutyltin compounds only dibutyltin dichloride had irritant properties while all tributyltin compounds tested were active. Tetrabutyltin did not cause a chemical burn after a single application on human skin.

CELLULAR AND BIOCHEMICAL ASPECTS OF ORGANOTIN TOXICITY

Effects on Mitochondrial Respiration

Already in the first investigation on the biochemical effects of organotin compounds, diethyltin dichloride and triethyltin sulphate were recognized as powerful metabolic inhibitors, each with a different mode of acton (Aldridge and Cremer, 1955).

Diethyltin and other dialkyltin homologs (from dimethyltin to dihexyltin) were found to inhibit oxygen and substrate consumption of isolated rat liver mitochondria (Aldridge, 1976; Penninks and Seinen, 1980). In the presence of dialkyltin compounds various substrates were not completely oxidized, but were accumulated as α-keto acids. Therefore, the dialkyltin compounds were proposed to inhibit the two α-keto acid oxidizing enzyme complexes in mitochondria, namely pyruvate and α-keto-glutarate dehydrogenase. Since dialkyltin compounds demonstrate a high chemical affinity for dithiol groups, this inhibition is suggested to be due to binding to lipoic acid or lipoyl dehydrogenase. These dithiol-containing molecules are essential factors in the oxidation of the α-keto acids (Aldridge, 1976; Penninks and Seinen, 1980). At higher concentrations, dibutyltin dichloride was also observed to inhibit the oxidative phosphorylation processes in mitochondria. Both an interaction with the ATP synthase complex (Cain et al., 1977) and an uncoupling effect have been described (Penninks et al., 1983).

The effects of the triorganotin compounds on mitochondria have been studied intensively (Aldridge and Cremer, 1955; Aldridge, 1958, 1976; Aldridge and Street, 1964; Selwyn et al., 1970; Selwyn, 1976). Three different types of interaction with the mitochondrial respiration could be distinguished. In halide-containing media, the triorganotin compounds mediated an exchange of halide for hydroxyl ions across the mitochondrial membranes, resulting in a disturbance of the existing proton gradient. The triorganotins were also found to bind to a component of the ATP synthase complex, leading to a direct inhibition of ATP production. Finally, gross mitochondrial swelling was noticed upon incubation of particularly the more lipophilic triorganotin compounds. As a result of these effects, the triorganotins act as effective inhibitors of mitochondrial ATP synthesis. For a series of trialkyltin compounds, the order of effectiveness in causing 50% inhibition of ATP production in isolated liver mitochondria was found to be triethyltin > tripropyltin > tributyltin > trihexyltin > trimethyltin (Aldridge, 1976).

Effects on Cellular Functions

Recently, comparable studies on the cellular effects of a series of dialkyltin and

trialkyltin chlorides were carried out, using isolated rat thymocytes (Penninks and Seinen, 1980, 1987; Snoeij et al., 1986a, c, d). Thymocytes incubated with dimethyltin, diethyltin, dibutyltin, or dioctyltin demonstrated a marked increase in the consumption of glucose and accumulated pyruvate and lactate in the cell. Diethyltin and dibutyltin were the most active homologs in this respect, affecting these parameters at concentrations as low at 10^{-6} M (Penninks and Seinen, 1980). In glucose-containing media, cellular ATP levels were not affected by dibutyltin dichloride, but decreased considerably when glucose was omitted from the medium (Penninks and Seinen, 1987). These findings can be explained by considering the inhibition of pyruvate dehydrogenase caused by dialkyltin compounds. Due to this action, the entrance of glycolytic end products into the TCA cycle is disturbed. As a consequence, pyruvate accumulates in the cell and is largely converted into lactate. As an adaptation to the reduced TCA cycle activity, the glycolytic pathway will be activated, leading to an increased glucose consumption. The subsequential increase in glycolytic phosphorylation is apparently capable of maintaining the intracellular ATP levels, provided that glucose is present in the incubation medium.

Besides effects on cell energetics, the dialkyltin compounds also interfere with the synthesis of DNA, RNA, and proteins in isolated rat thymocytes. Micromolar concentrations of diethyltin or dibutyltin effectively decreased the incorporation of DNA and protein precursors, while the incorporation of uridine into RNA was increased by these dialkyltins (Miller et al., 1980; Penninks and Seinen, 1987). Dibutyltin and dioctyltin compounds also affected the proliferation of cultured rabbit chondrocytes. At the micromolar level these compounds inhibited the incorporation of a DNA precursor, while at lower concentrations a stimulation of DNA synthesis was noticed (Webber et al., 1985). Whether the antiproliferative effects of the dialkyltin compounds are related to the inhibition of mitochondrial respiration is not known.

Within the series of trialkyltin chlorides, all compounds exhibit marked effects on thymocyte energetics, except for the most hydrophilic and lipophilic homologs, trimethyltin and trioctyltin, respectively (Snoeij et al., 1986d). Incubation of rat thymocytes with concentrations higher than 10^{-7} M of the active trialkyltin compounds resulted in an increase of glucose consumption and a marked accumulation of lactate. Pyruvate levels, however, were only slightly raised. The intracellular ATP levels were drastically reduced, especially when glucose was omitted from the medium (Snoeij et al., 1986c, d). These effects can be explained in view of the well-studied mode of action at the mitochondrial level. The trialkyltin compounds inhibit ATP formation by binding to the ATP synthase complex and by mediating an ion exchange across the membranes of mitochondria. The latter phenomenon results in an inhibition of pyruvate transport across the mitochondrial membranes. As a consequence, pyruvate will accumulate in the cytoplasm. Due to the inhibition of the oxidative phosphorylation also NADH will accumulate. These two products induce a shift in the balance between pyruvate and lactate in the cytoplasm. As a result, virtually all pyruvate will be converted into lactate at the expense of cytosolic NADH. The glycolysis, with only a limited production of ATP, is thereby stimulated to continue. Therefore glucose con-

sumption is increased, but in contrast to the dialkyltin compounds, the increased aerobic glycolysis does not provide enough ATP to meet the demands of trialkyltin-exposed cells.

A correlation was noticed between the suppression of the cellular energy state and the inhibition of macromolecular synthesis and cell proliferation caused by trialkyltin compounds. The incorporation of DNA, RNA, and protein precursors and also the production of cyclic AMP were markedly diminished in thymocytes by those trialkyltin homologs that also interfered with energy metabolism (Snoeij *et al.*, 1986c, d). Antiproliferative effects of trialkyltin compounds were also described for a baby hamster kidney cell line (Reinhardt *et al.*, 1982), for rabbit chondrocytes (Webber *et al.*, 1985), and for rat skin, either in organ culture or *in vivo* (Kao *et al.*, 1983; Middleton and Pratt, 1978). This property of the triorganotin compounds may very well be the cause of the drastic effects of TBTO on embryonic development of explants of mice *in vitro*, as was studied recently using limb bud organ cultures (Krowke *et al.*, 1986).

Rat brain slices were originally used to study the effects of triethyltin at the cellular level (Cremer, 1957). As with thymocytes the consumption of glucose and the production of lactate were increased in triethyl-exposed brain slices. However, pyruvate levels were considerably decreased. Also in brain slices of rats given a single dose of triethyltin *in vivo*, an increase in lactate to pyruvate ratios was observed. Liver and kidney slices of these rats did not differ from control slices in this respect.

At concentrations much higher than those disturbing mitochondrial respiration, the triorganotin compounds exhibit severe membrane-damaging properties toward various cell species. In a concentration range of 10^{-5} to 2×10^{-4} M, the lipophilic homologs, tripropyltin, tributyltin, triphenyltin, and tricyclohexyltin caused lysis of red blood cells of many animal species (Byington *et al.*, 1974; Snoeij *et al.*, 1986a). At even lower concentrations (10^{-6} to 10^{-5} M), membrane integrity of rat bone marrow cells and rat thymocytes was affected by tributyltin (Snoeij *et al.*, 1986a). Compared to these cells, isolated rat hepatocytes appeared more resistant to the cytotoxic properties of tributyltin (Snoeij *et al.*, 1986b).

MECHANISMS OF ACTION

The neurotoxic properties of the organotin compounds are limited to the lower homologs of the tetraalkyltin and trialkyltin compounds. Since the symptoms of tetramethyltin or tetraethyltin intoxication were identical to those of the respective trialkyltin compounds (Barnes and Stoner, 1959), the tetraalkyltins were suggested to become neurotoxic upon dealkylation *in vivo* (Cremer, 1958). Although triethyltin shows a high affinity for myelin *in vitro* (Lock and Aldridge, 1977), this compound was not found to accumulate in the CNS (Cremer, 1957; Rose and Aldridge, 1968). Moreover, the small amount of triethyltin taken up by the brain demonstrated a homogeneous distribution pattern. Fractions rich in white matter did not accumulate the compound specifically (Rose and Aldridge, 1968; Cook *et al.*, 1984a). The property of triethyltin to disturb mitochondrial respiration *in vitro* has been suggested to cause a cytotoxic hypoxia in the CNS (Reiter *et al.*, 1981). Indeed, the energy metabolism of brain slices, prepared from rats treated

with triethyltin *in vivo,* was found to be disturbed. However, both gray and white matter were affected and it is therefore not clear whether the biochemical lesion is related to the pathological one (Cremer, 1957; McMillan and Wenger, 1985). Although triethyltin altered neurotransmitter levels in adult rats, a chronic exposure study using young rats revealed long-term behavioral changes without disturbing neurotransmitter levels (Hanin *et al.,* 1984). Therefore, none of the described effects at the cellular level seem to explain the triethyltin-induced neurotoxicity at present.

Recently, a pathogenetic mechanism was proposed for the neurologic damage observed in mammals intoxicated with trimethyltin (Chang, 1986). Necrosis of hippocampal neurons is most likely caused by a delicate disturbance of the electrical circuits between the two major hippocampal neuron formations, the Ammon's horn and the dentate gyrus. Trimethyltin is thought to reduce the inhibitory control of the granule cells in the dentate gyrus, resulting in hyperexcitation and subsequent necrosis of the pyramidal cells in the Ammon's horn (Dyer and Boyes, 1984; Chang and Dyer, 1985). However, a molecular mechanism for the trimethyltin-induced necrosis is not available. Although trimethyltin showed profound effects on the levels of neurotransmitters and phosphoproteins in the CNS, alterations were found several days after exposure to trimethyltin (Valdes *et al.,* 1983; DeHaven *et al.,* 1984; Hanin *et al.,* 1984; O'Callaghan and Miller, 1984; Wilson *et al.,* 1986). In addition, neurochemical changes were sometimes secondary to neuronal damage (Valdes *et al.,* 1983; Naalsund *et al.,* 1985) and were frequently observed in areas different from those known to be histologically affected (Doctor *et al.,* 1982; Hanin *et al.,* 1984). In contrast to these relatively late effects, electrical activity of the hippocampus was already changed within 2 hr after trimethyltin exposure (Dyer and Boyes, 1984). Trimethyltin did not accumulate in the brain, and the amount of tin that was recovered from the brain of trimethyltin-exposed rats did not reveal a regional-specific distribution (Cook *et al.,* 1984b; Harry *et al.,* 1985). Since trimethyltin was a weak inhibitor of mitochondrial respiration *in vitro* (Aldridge, 1976) and appeared only mildly cytotoxic to isolated cells (Snoeij *et al.,* 1986a), none of the known interactions at the mitochondrial and cellular level seem to provide an explanation for the mode of action. In recent studies, trimethyltin was found to reduce the hippocampal zinc concentration (Chang and Dyer, 1984) and adrenal levels of epinephrine and norepinephrine (Ally *et al.,* 1986). Since both effects were noticed prior to the onset of neurologic damage, they may become important in the elucidation of the mechanism underlying the trimethyltin-induced neurotoxicity.

The induction of bile duct lesions and subsequent hepatotoxicity is a special feature of the intermediate dialkyltin homologs (diethyltin to dihexyltin). Tributyltin compounds can also induce this type of injury, but probably only after conversion to dibutyltin. The occurrence of bile duct lesions is restricted to those animal species which have a combined bile and pancreatic duct and is correlated with the presence of a tin compound in the bile. From unpublished observations, we inferred that tin must be present in the form of a dialkyltin compound in order to cause this lesion. After a single oral dose of either tributyltin or dibutyltin to rats, only the latter compound appeared in the bile. When trioctyltin or dioctyltin

—compounds that do not induce bile duct lesions—were given to rats, no dialkyltin was detectable in the bile. That bile and pancreatic juices are essential in the actual development of the lesion was already shown by Magee *et al.* (1957), but the precise mechanism of the inflammatory reaction is still unknown.

Immunosuppression is observed in rats upon exposure to various dialkyltin compounds, particularly dibutyltin and dioctyltin. Oral treatment of rats with tributyltin but not trioctyltin also caused immunotoxic effects. In recent studies the effects of a single oral dose of tributyltin, dibutyltin, and monobutyltin on the thymus of rats were compared. The process of thymus involution as judged by histology, pool sizes of different thymocyte subpopulations, and their proliferative capacity was similar for tributyltin and dibutyltin. Both compounds induced a selective elimination of thymic lymphoblasts, resulting in a marked depletion of small-sized, nonproliferative lymphocytes a few days later. With the reappearance of actively dividing lymphoblasts recovery initiated and was complete 9 days after dosing. Monobutyltin, however, did not affect thymus weights under identical conditions (Snoeij, 1987). Intravenous administration of these compounds revealed that only dibutyltin was capable of producing thymus atrophy via this route. The oral and intravenous data suggest a conversion of tributyltin to dibutyltin upon oral exposure. In subsequent studies, using radiolabeled tributyltin, this suggestion was verified. A few hours after a single oral dose, the parent compound and its dealkylated metabolites dibutyltin and monobutyltin were found in the blood of rats. Therefore, it was concluded that dibutyltin is responsible for the thymus atrophy and subsequent immunotoxicity observed in rats orally exposed to tri- and dibutyltin compounds (Snoeij, 1987).

The finding that the immunotoxic action declined within the series of trialkyltin compounds from tributyltin to trioctyltin (Snoeij *et al.*, 1985) may be explained by a limited absorption or metabolism of the higher trialkyltin homologs.

Indirect effects of organotin compounds on bone marrow or adrenal glands do not seem to be important in the involution of the thymus. Neither the spontaneous blastogenesis nor the colony formation of bone marrow cells isolated from dibutyltin-treated mice was affected (Penninks *et al.*, 1985). Stress-mediated release of glucocorticoids was not essential for the thymus atrophy in rats fed dioctyltin or tributyltin (Seinen and Willems, 1976; Snoeij *et al.*, 1985). Histologic studies did not give the impression that thymus atrophy is the result of a dysfunction of the thymic reticuloepithelial cells (Penninks *et al.*, 1985; Evans *et al.*, 1986). Therefore, a direct antiproliferative effect, which has been observed *in vivo* and *in vitro* seems to be important in the mode of action of the dialkyltin-induced thymus atrophy.

The mechanism of the cutaneous toxicity of tributyltin and dibutyltin compounds can be explained on the basis of their cytotoxicity. When rat skin was exposed to relatively high concentrations of tributyltin, the energy metabolism and proliferation of epidermal cells were disturbed. ATP levels, oxygen consumption, and DNA synthesis were decreased, while lactate was found to accumulate (Middleton and Pratt, 1978; Middleton, 1982). Inhibition of oxidative metabolism and cell proliferation preceded histologic signs of epidermal necrosis. Low concentrations of dibutyltin and tributyltin compounds applied on the skin

of rats caused an increase in DNA synthesis. This was explained to be the proliferative response of a small number of epidermal cells, as a reaction to overcome minimal damage (Middleton and Pratt, 1978).

CONCLUDING REMARKS

At present, the relation between the well-studied biochemical properties of the organotin compounds and the effects observed at the cellular level is about to be elucidated. With the possible exception of cutaneous toxicity, the relation between the biochemical properties and target organ toxicity in mammals is still very elusive. For a better understanding of the apparently complex mechanisms underlying the neurotoxic, immunotoxic, and hepatotoxic effects, more investigations concerning the absorption, distribution, and metabolism of the organotin compounds, but also of the structure and function of the target organs, are necessary.

The biochemical and cellular disturbances are possibly more easily related to the toxic action of organotin compounds on the less complex aquatic biota. Therefore, the knowledge obtained from the various studies with isolated mammalian cells may be of value in the current investigations on the aquatic toxicity of the organotin compounds.

REFERENCES

Alajouanine, Th., Dérobert, L., and Thiéffry, S. (1958). Etude clinique d'ensemble de 210 cas d'intoxication par les sels organiques d'etain. *Rev. Neurol.* **98**, 85–96.

Aldridge, W. N. (1958). The biochemistry of organotin compounds. Trialkyltins and oxidative phosphorylation. *Biochem. J.* **69**, 367–376.

Aldridge, W. N. (1976). The influence of organotin compounds on mitochondrial functions. *Adv. Chem. Ser.* **157**, 186–196.

Aldridge, W. N., and Cremer, J. E. (1955). The biochemistry of organotin compounds. Diethyltin dichloride and triethyltin sulphate. *Biochem. J.* **61**, 406–418.

Aldridge, W. N., and Street, B. W. (1964). Oxidative phosphorylation. Biochemical effects and properties of trialkyltins. *Biochem. J.* **91**, 287–297.

Ally, A. I., Vieira, L., and Reuhl, K. R. (1986). Trimethyltin as a selective adrenal chemosympatholytic agent *in vivo:* Effect precedes both clinical and histopathological evidence of toxicity. *Toxicology* **40**, 215–229.

Alzieu, C., and Heral, M. (1984). Ecotoxicological effects of organotin compounds on oyster culture. *In* "Ectoxicological Testing for the Marine Environment" (G. Persoone *et al.,* Eds.), Vol. 2, pp. 187–196. State University of Ghent, Ghent, and Institute for Marine Scientific Research, Bredene, Belgium.

Barnes, J. M., and Magee, P. N. (1958). The biliary and hepatic lesion produced experimentally by dibutyltin salts. *J. Pathol. Bacteriol.* **75**, 267–279.

Barnes, J. M., and Stoner, H. B. (1958). Toxic properties of some dialkyl and trialkyltin salts. *Brit. J. Ind. Med.* **15**, 15–22.

Barnes, J. M., and Stoner, H. B. (1959). The toxicology of tin compounds. *Pharmacol. Rev.* **11**, 211–232.

Beaumont, A. R., and Budd, M. D. (1984). High mortality of the larvae of the common mussel at low concentrations of tributyltin. *Mar. Pollut. Bull.* **15**, 402–405

Bentue-Ferrer, D., Reymann, J. M., Van den Driessche, J., Allain, H., and Bagot, H. (1985). Effect of triethyltin chloride on the central aminergic neurotransmitters and their metabolites: Relationship with pathophysiology of aging. *Exp. Aging Res.* **11**, 137–141.

Bierkamper, G. G., and Bassett, D. J. P. (1984). Is triethyltin-induced muscular dysfunction a result of

mitochondrial compromise? *In* "Cellular and Molecular Neurotoxicology" (T. Narahashi, Ed.), pp. 109–121. Raven Press, New York.

Bouldin, T. W., Goines, N. D., Bagnell, C. R., and Krigman, M. R. (1981). Pathogenesis of trimethyltin neuronal toxicity. Ultrastructural and cytochemical observations. *Amer. J. Pathol.* **104**, 237–249.

Bouldin, T. W., Goines, N. D., and Krigman, M. R. (1984). Trimethyltin retinopathy. Relationship of subcellular response to neuronal subspecialization. *J. Neuropathol. Exp. Neurol.* **43**, 162–174.

Brown, A. W., Aldridge, W. N., Street, B. W., and Verschoyle, R. D. (1979). The behavioural and neuropathologic sequelae of intoxication by trimethyltin compounds in the rat. *Amer. J. Pathol.* **97**, 59–82.

Brown, A. W., Verschoyle, R. D., Street, B. W., Aldridge, W. N., and Grindley, H. (1984). The neurotoxicity of trimethyltin chloride in hamsters, gerbils and marmosets. *J. Appl. Toxicol.* **4**, 12–21.

Byington, K. H., Yeh, R. Y., and Forte, L. R. (1974). The hemolytic activity of some trialkyltin and triphenyltin compounds. *Toxicol. Appl. Pharmacol.* **27**, 230–240.

Cain, K., Hyams, R. L., and Griffiths, D. E. (1977). Studies on the energy-linked reactions: Inhibition of oxidative phosphorylation and energy-linked reactions by dibutyltin dichloride. *FEBS. Lett.* **82**, 23–28.

Cardarelli, N. F. (1976). "Controlled-Release Pesticide Formulations." CRC Press, Cleveland, OH.

Chang, L. W. (1986). Neuropathology of trimethyltin: A proposed pathogenetic mechanism. *Fundam. Appl. Toxicol.* **6**, 217–232.

Chang, L. W., and Dyer, R. S. (1983). Trimethyltin-induced pathology in sensory neurons. *Neurobehav. Toxicol. Teratol.* **5**, 673–696.

Chang, L. W., and Dyer, R. S. (1984). Trimethyltin induced zinc depletion in rat hippocampus. *In* "The Neurobiology of Zinc" (C. Frederickson and G. Howell, Eds.), Vol. 2, pp. 275–290. Alan R. Liss Series in Neurology and Neurobiology, A. R. Liss, New York.

Chang, L. W., and Dyer, R. S. (1985). Early effects of trimethyltin on the dentate gyrus basket cells: A morphological study. *J. Toxicol. Environ. Health* **16**, 641–653.

Chang, L. W., Tiemeyer, T. M., Wenger, G. R., McMillan, D. E., and Reuhl, K. R. (1982a). Neuropathology of trimethyltin intoxication. I. Light microscopy study. *Environ. Res.* **29**, 435–444.

Chang, L. W., Tiemeyer, T. M., Wenger, G. R., McMillan, D. E., and Reuhl, K. R. (1982b). Neuropathology of trimethyltin intoxication. II. Electron microscopic study on the hippocampus. *Environ. Res.* **29**, 445–458.

Chang, L. W., Tiemeyer, T. M., Wenger, G. R., and McMillan, D. E. (1983a). Neuropathology of trimethyltin intoxication. III. Changes in the brainstem neurons. *Environ. Res.* **30**, 399–411.

Chang, L. W., Wenger, G. R., and McMillan, D. E., and Dyer, R. S. (1983b). Species and strain comparison of acute neurotoxic effects of trimethyltin in mice and rats. *Neurobehav. Toxicol. Teratol.* **5**, 337–350.

Chang, L. W., Wenger, G. R., and McMillan, D. E. (1984). Neuropathology of trimethyltin intoxication. IV. Changes in the spinal cord. *Environ. Res.* **34**, 123–134.

Chliamovitch, Y.-P., and Kuhn, C. (1977). Behavioral, haematological and histological studies on acute toxicity of bis(tri-*n*-butyltin) oxide on *Salmo gairdneri* Richardson and *Tilapia rendalli* Boulenger. *J. Fish Biol.* **10**, 575–585.

Cleary, J. J., and Stebbing, A. R. D. (1985). Organotin and total tin in coastal waters of southwest England. *Mar. Pollut. Bull.* **16**, 350–355.

Cook, L. L., Stine Jacobs, K., and Reiter, L. W. (1984a). Tin distribution in adult and neonatal rat brain following exposure to triethyltin. *Toxicol. Appl. Pharmacol.* **72**, 75–81.

Cook, L. L., Stine, K. E., and Reiter, L. W. (1984b). Tin distribution in adult rat tissues after exposure to trimethyltin and triethyltin. *Toxicol. Appl. Pharmacol.* **76**, 344–348.

Cremer, J. E. (1957). The metabolism *in vitro* of tissue slices from rats given triethyltin compounds. *Biochem. J.* **67**, 87–96.

Cremer, J. E. (1958). The biochemistry of organotin compounds. The conversion of tetraethyltin into triethyltin in mammals. *Biochem. J.* **68**, 685–692.

DeHaven, D. L., Walsh, T. J., and Mailman, R. B. (1984). Effects of trimethyltin on dopaminergic and serotonergic function in the central nervous system. *Toxicol. Appl. Pharmacol.* **75**, 182–189.

Doctor, S. V., Costa, L. G., Kendall, D. A., and Murphy, S. D. (1982). Trimethyltin inhibits uptake of neurotransmitters into mouse forebrain synaptosomes. *Toxicology* 25, 213–221.

Duncan, J. (1980). The toxicology of molluscicides. The organotins. *Pharmacol. Ther.* 10, 407–429.

Dyer, R. S., and Boyes, W. K. (1984). Trimethyltin reduces recurrent inhibition in rats. *Neurobehav. Toxicol. Teratol.* 6, 369–371.

Dyer, R. S., Howell, W. E., and Reiter, L. W. (1981). Neonatal triethyltin exposure alters adult electrophysiology in rats. *Neurotoxicology* 2, 609–623.

Evans, J. G., Scott, M. P., and Miller, K. (1986). The effect of pregnancy on dioctyltin dichloride-induced thymic injury. *Thymus* 8, 319–320.

Fechter, L. D., Young, J. S., and Nuttall, A. L. (1986). Trimethyltin ototoxicity. Evidence for a cochlear site of injury. *Hear. Res.* 23, 275–282.

Fortemps, E., Amand, G., Bomboir, A., Lauwerys, R., and Laterre, E. C. (1978). Trimethyltin poisoning. Report on two cases. *Int. Arch. Occup. Environ. Health* 41, 1–6.

Gerren, R. A., Groswald, D. E., and Luttges, M. W. (1976). Triethyltin toxicity as a model for degenerative disorders. *Pharmacol. Biochem. Behav.* 5, 299–307.

Graham, D. I., Bonilla, E., Gonatas, N. K., and Schotland, D. L. (1976a). Core formation in the muscles of rats intoxicated with triethyltin sulfate. *J. Neuropathol. Exp. Neurol.* 35, 1–13.

Graham, D. I., DeJezus, P. V., Pleasure, D. E., and Gonatas, N. K. (1976b). Triethyltin sulfate-induced neuropathy in rats. *Arch. Neurol.* 33, 40–48.

Hall, L. W., and Pinkney, A. E. (1985). Acute and sublethal effects of organotin compounds on aquatic biota: an interpretative literature evaluation. *CRC Toxicol.* 14, 159–209.

Hanin, I., Krigman, M. R., and Mailman, R. B. (1984). Central neurotransmitter effects of organotin compounds: Trials, tribulations and observations. *Neurotoxicology* 5, 267–278.

Harry, G. J., Goodrum, J. F., Krigman, M. R., and Morell, P. (1985). The use of synapsin I as a biochemical marker for neuronal damage by trimethyltin. *Brain Res.* 326, 9–18.

Harry, G. J., and Tilson, H. A. (1981). The effect of postpartum exposure to triethyltin on the neurobehavioral functioning of rats. *Neurotoxicology* 2, 283–296.

Hodge, V. F., Seidel, S. L., and Goldberg, E. D. (1979). Determination of tin (IV) and organotin compounds in natural waters, coastal sediments and macro algae by atomic absorption spectrometry. *Anal. Chem.* 51, 1256–1259.

Hulström, D., Forssén, M., Petterson, A., Tengvar, C., Jarild, M., and Olsson, Y. (1984). Vascular permeability in acute triethyltin-induced brain edema studied with FITC-dextrans, sodium fluorescein and horseradish peroxidase as tracers. *Acta Neurol. Scand.* 69, 255–263.

Ishaaya, I., Engel, J. L., and Casida, J. E. (1976). Dietary triorganotins affect lymphatic tissues and blood composition of mice. *Pestic. Biochem. Physiol.* 6, 270–279.

Iwai, H., and Wada, O. (1981), Dealkylation of tetraalkyltin compounds in the intestinal mucosa of rabbits. *Ind. Health* 19, 247–253.

Kaars Sijpesteijn, A., Rijkens, F., Luijten, J. G. A., and Willemsen, L. W. (1962). On the antifungal and antibacterial activity of some tri-substituted organogermanium, organotin and organolead compounds. *Antonie van Leeuwenhoek* 28, 346–356.

Kao, J., Hall, J., and Holland, J. M. (1983). Quantitation of cutaneous toxicity: An *in vitro* approach using skin organ culture. *Toxicol. Appl. Pharmacol.* 68, 206–217.

Kimbrough, R. D. (1976). Toxicity and health effects of selected organotin compounds: A review. *Environ. Health Perspect.* 14, 51–56.

Krajnc, E. I., Wester, P. W., Loeber, J. G., Van Leeuwen, F. X. R., Vos, J. G., Vaessen, H. A. M. G., and Van der Heijden, C. A. (1984). Toxicity of bis(tri-*n*-butyltin)oxide in the rat. I. Short-term effects on general parameters and on the endocrine and lymphoid systems. *Toxicol. Appl. Pharmacol.* 75, 363–386.

Krowke, R., Bluth, U., and Neubert, D. (1986). *In vitro* studies on the embryotoxic potential of bis(tri-*n*-butyltin)oxide in a limb bud organ culture system. *Arch. Toxicol.* 58, 125–129.

Laughlin, R., and Lindén, O. (1985). Fate and effects of organotin compounds. *Ambio* 14, 88–94.

Laughlin, R., Nordlund, K., and Lindén, O. (1984). Long-term effects of tributyltin compounds on the baltic amphipod, *Gammarus oceanicus. Mar. Environ. Res.* 12, 243–271.

Leow, A. C. T., Anderson, R. McD., Little, R. A., Leaver, D. D. (1979). A sequential study of

changes in the brain and cerebrospinal fluid of the rat following triethyltin poisoning. *Acta Neuropathol.* **47,** 117–121.

Lewis, W. R., and Hedges, E. S. (1957). Applications of organotin compounds. *Adv. Chem. Ser.* **23,** 190–203.

Lock, E. A., and Aldridge, W. N. (1977). The binding of triethyltin to rat brain myelin. *J. Neurochem.* **25,** 871–876.

Löwig, C. (1852). Ueber Zinn-aethyle, neu aus Zinn und Aethyl bestehende organische Radicale. *J. Prakt. Chem.* **57,** 385.

Luijten, J. G. A. (1971). Applications and biological effects or organotin compounds. *In* "Organotin Compounds" (A. K. Sawyer, Ed.), pp. 931–974. Dekker, New York.

Lyle, W. H. (1958). Lesions of the skin in process workers caused by contact with butyl tin compounds. *Brit. J. Ind. Med.* **15,** 193–196.

Magee, P. N., Stoner, H. B., and Barnes, J. M. (1957). The experimental production of oedema in the central nervous system of the rat by triethyltin compounds. *J. Pathol. Bacteriol.* **73,** 107–124.

Maguire, R. J., Chau, Y. K., Bengert, G. A., Hale, E. J., Wong, P. T. S., and Kramar, O. (1982). Occurrence of organotin compounds in Ontario lakes and rivers. *Environ. Sci. Technol.* **16,** 698–702.

Maguire, R. J., Tkaez, R. J., Chau, Y. K., Bengert, G. A., and Wong, P. T. S. (1986). Occurrence of organotin compounds in water and sediment in Canada. *Chemosphere* **15,** 253–274.

McMillan, D. E., and Wenger, G. R. (1985). Neurobehavioral toxicology of trialkyltins. *Pharmacol. Rev.* **37,** 365–379.

Merkord, J., Hennighausen, G., Karnstedt, U., and Lange, P. (1982). Die biliäre Exkretion von Organozinn bei der narkotisierten Ratte nach Gabe von Di-*n*-alkylzinndichloriden mit unterschiedlicher Länge der Alkylketten. *Pharmazie* **37,** 146.

Middleton, M. C. (1982). Early metabolic changes in rat epidermis and dermis following cutaneous application of irritant doses of tributyltin. *J. Invest. Dermatol.* **79,** 163–166.

Middleton, M. C., and Pratt, I. (1978). Changes in incorporation of ^{3}H-thymidine into DNA of rat skin following cutaneous application of dibutyltin, tributyltin and 1-chloro-2:4-dinitrobenzene and the relationship of these changes to a morphological assessment of the cellular damage. *J. Invest. Dermatol.* **71,** 305–310.

Miller, K., Scott, M. P., and Foster, J. R. (1983). Thymic involution in rats given diets containing dioctyltin dichloride. *Clin. Immunol. Immunopathol.* **30,** 62–70.

Miller, R. R., Hartung, R., and Cornish, H. H. (1980). Effects of diethyltin dichloride on amino acids and nucleoside transport in suspended rat thymocytes. *Toxicol. Appl. Pharmacol.* **55,** 564–571.

Moore, K. E., and Brody, T. M. (1961). The effect of triethyltin on tissue amines. *J. Pharmacol. Exp. Ther.* **132,** 6–12.

Mueller, M. D. (1984). Tributyltin detection at trace levels in water and sediments using G.C. with flame-photometric detection and GC-MS. *Fresenius Z. Anal. Chem.* **317,** 32–36.

Mushak, P., Krigman, M. R., and Mailman, R. B. (1982). Comparative organotin toxicity in the developing rat: Somatic and morphological changes and relationship to accumulation of total tin. *Neurobehav. Toxicol. Teratol.* **4,** 209–215.

Naalsund, L. U., Allen, C. N., and Fonnum, F. (1985). Changes in neurobiological parameters in the hippocampus after exposure to trimethyltin. *Neurotoxicology* **6,** 145–158.

O'Callaghan, J. P., and Miller, D. B. (1984). Neuronspecific phosphoproteins as biochemical indicators of neurotoxicity: Effects of acute administration of trimethyltin to the adult rat. *J. Pharmacol. Exp. Ther.* **231,** 736–743.

O'Callaghan, J. P., Miller, D. B., and Reiter, L. W. (1983). Acute postnatal exposure to triethyltin in the rat: Effects on specific protein composition of subcellular fractions from developing and adult brain. *J. Pharmacol. Exp. Ther.* **224,** 466–472.

Pelikan, Z. and Cerny, E. (1970). Toxic effects of some mono-*n*-butyltin compounds on white mice. *Arch. Toxikol.* **27,** 79–84.

Penninks, A. H., Kuper, F., Spit, B. J., and Seinen, W. (1985). On the mechanism of dialkyltin induced thymus involution. *Immunopharmacology* **10,** 1–10.

Penninks, A. H., and Seinen, W. (1980). Toxicity of organotin compounds. IV. Impairment of energy

metabolism of rat thymocytes by various dialkyltin compounds. *Toxicol. Appl. Pharmacol.* **56,** 221–231.

Penninks, A. H., and Seinen, W. (1987). Immunotoxicity of organotin compounds. A cell biological approach to dialkyltin-induced thymus atrophy. *In* "Immunotoxicology. Proceedings of the International Symposium on the Immunological System as Target for Toxic Damage" (A. Berlin *et al.,* Eds.), pp. 258–278. Nijhoff, Hingham, MA.

Penninks, A. H., Verschuren, P. M., and Seinen, W. (1983). Di-*n*-butyltin dichloride uncouples oxidative phosphorylation in rat liver mitochondria. *Toxicol. Appl. Pharmacol.* **70,** 115–120.

Reinhardt, C. A., Schawalder, H., and Zbinden, G. (1982). Cell detachment and cloning efficiency as parameters for cytotoxicity. *Toxicology* **25,** 47–52.

Reiter, L. W., Haevner, G. B., Dean, K. F., and Ruppert, P. H. (1981). Developmental and behavioral effects of early postnatal exposure to triethyltin in rats. *Neurobehav. Toxicol. Teratol.* **3,** 285–293.

Reuhl, K. R., Gilbert, S. G., Mackenzie, B. A., Mallett, J. E., and Rice, D. C. (1985). Acute trimethyltin intoxication in the monkey (*Macaca fascicularis*). *Toxicol. Appl. Pharmacol.* **79,** 436–452.

Rose, M. S., and Aldridge, W. N. (1968). The interaction of triethyltin with components of animal tissues. *Biochem. J.* **106,** 821–828.

Ross, A. (1965). Industrial application of organotin compounds. *Ann. N.Y. Acad. Sci.* **125,** 107–123.

Ross, W. D., Emmett, E. A., Steiner, J., and Tureen, R. (1981). Neurotoxic effects of occupational exposure to organotins. *Amer. J. Psychiatry* **138,** 1092–1095.

Seinen, W. (1980). Immunosuppression induced by certain organotin compounds. *Vet. Sci. Commun.* **3,** 279–287.

Seinen, W., Helder, Th., Vernij, H., Penninks, A. H., and Leeuwangh, P. (1981). Short-term toxicity of tri-*n*-butyltin chloride in rainbow trout (*Salmo gairdneri* Richardson) yolk sac fry. *Sci. Total Environ.* **19,** 155–166.

Seinen, W., and Penninks, A. H. (1979). Immune suppression as a consequence of a selective cytotoxic activity of certain organometallic compounds on thymus and thymus-dependent lymphocytes. *Ann. N.Y. Acad. Sci.* **320,** 499–517.

Seinen, W., and Willems, M. I. (1976). Toxicity of organotin compounds. I. Atrophy of thymus and thymus-dependent lymphoid tissue in rats fed di-*n*-octyltin dichloride. *Toxicol. Appl. Pharmacol.* **35,** 63–75.

Seinen, W., Vos, J. G., Van Spanje, I., Snoek, M., Brands, R., and Hooykaas, H. (1977a). Toxicity of organotin compounds. II. Comparative *in vivo* and *in vitro* studies with various organotin and organolead compounds in different animal species with special emphasis on lymphocyte cytotoxicity. *Toxicol. Appl. Pharmacol.* **42,** 197–212.

Seinen, W., Vos, J. G., Van Krieken, R., Penninks, A. H., Brands, R., and Hooykaas, H. (1977b). Toxicity of organotin compounds. III. Suppression of thymus-dependent immunity in rats by di-*n*-butyltin dichloride. *Toxicol. Appl. Pharmacol.* **42,** 213–224.

Seligman, P. F., Valkirs, A. O., and Lee, R. F. (1986). Degradation of tributyltin in San Diego Bay, California, Waters. *Environ. Sci. Technol.* **20,** 1229–1235.

Selwyn, M. J. (1976). Triorganotin compounds as ionophores and inhibitors of ion translocating ATPases. *Adv. Chem. Ser.* **157,** 204–226.

Selwyn, M. J., Dawson, A. P., Stockdale, M., and Gains, N. (1970). Chloride–hydroxide exchange across mitochondrial, erythrocyte and artificial lipid membranes, mediated by trialkyl- and triphenyltin compounds. *Eur. J. Biochem.* **14,** 120–126.

Sheldon, A. W. (1975). Effects of organotin anti-fouling coatings on man and his environment. *J. Paint Technol.* **47,** 54–58.

Smith, M. E. (1973). Studies on the mechanism of demyelination: Triethyltin-induced demyelination. *J. Neurochem.* **21,** 357–372.

Smith, P. J. (1978). "Toxicological Data on Organotin Compounds." Int. Tin Research Institute, no. 538.

Snoeij, N. J. (1987). "Triorganotin Compounds in Immunotoxicology and Biochemistry." Ph.D. thesis, University of Utrecht.

Snoeij, N. J., Van Iersel, A. A. J., Penninks, A. H., and Seinen, W. (1985). Toxicity of triorganotin

compounds: Comparative *in vivo* studies with a series of trialkyltin compounds and triphenyltin chloride in male rats. *Toxicol. Appl. Pharmacol.* 81, 274–286.

Snoeij, N. J., Van Iersel, A. A. J., Penninks, A. H., and Seinen, W. (1986a). Triorganotin-induced cytotoxicity to rat thymus, bone marrow and red blood cells as determined by several *in vitro* assays. *Toxicology* 39, 71–83.

Snoeij, N. J., Van Iersel, A. A. J., Penninks, A. H., and Seinen, W. (1986b). Triorganotin-induced cytotoxicity to rat thymocytes. *Food Chem. Toxicol.* 24, 599–600.

Snoeij, N. J., Punt, P. M., Penninks, A. H., and Seinen, W. (1986c). Effects of tri-*n*-butyltin chloride on energy metabolism, macromolecular synthesis, precursor uptake and cyclic AMP production in isolated rat thymocytes. *Biochim. Biophys. Acta* 852, 234–243.

Snoeij, N. J., Van Rooijen, H. J. M., Penninks, A. H., and Seinen, W. (1986d). Effects of various inhibitors of oxidative phosphorylation on energy metabolism, macromolecular synthesis and cyclic AMP production in isolated rat thymocytes. *Biochim. Biophys. Acta* 852, 244–253.

Squibb, R. E., Carmichael, N. G., and Tilson, H. A. (1980). Behavioral and neuromorphological effects of triethyltin bromide in adult rats. *Toxicol. Appl. Pharmacol.* 55, 188–197.

Stebbing, A. R. D. (1985). Organotin and water quality—some lessons to be learned. *Mar. Pollut. Bull.* 16, 383–390.

Stoner, H. B. (1966). Toxicity of triphenyltin. *Brit. J. Ind. Med.* 23, 222–229.

Stoner, H. B., Barnes, J. M., and Duff, J. I. (1955). Studies on the toxicity of alkyltin compounds. *Brit. J. Pharmacol.* 10, 16–25.

Thain, J. E. (1983). "The Acute Toxicity of Bis(tributyltin)oxide to the Adults and Larvae of Some Marine Organisms." ICES paper CM 1983/E:13 (mimeograph). International Council for the Exploration of the Sea, Copenhagen.

Thain, J. E., and Waldock, M. J. (1986). The impact of tributyltin (TBT) antifouling paints on molluscan fisheries. *Water. Sci. Technol.* 18, 193–202.

Tugrul, S., Balkas, T. I., and Goldberg, E. D. (1983). Methyltins in the marine environment. *Mar. Pollut. Bull.* 14, 297–303.

U'ren, S. C. (1983). Acute toxicity of bis(tributyltin)oxide to a marine copepod. *Mar. Pollut. Bull.* 14, 303–306.

Valdes, J. J., Mactutus, C. F., Santos-Anderson, R. M., Dawson, R., and Annau, Z. (1983). Selective neurochemical and histological lesions in rat hippocampus following chronic trimethyltin exposure. *Neurobehav. Toxicol. Teratol.* 5, 357–361.

Van der Kerk, G. J. M. (1978). The organic chemistry of tin. *Chem. Tech.* 8, 356–365.

Van der Kerk, G. J. M., and Luijten, J. G. A. (1954). Investigations on organotin compounds. III. The biocidal properties of organotin compounds. *J. Appl. Chem.* 4, 314–319.

Van der Kerk, G. J. M., Luijten, J. G. A., and Noltes, J. G. (1958). Neuere Ergebnisse der Organo-Zinn Forschung. *Angew. Chem.* 70, 298–306.

Verschuuren, H. G., Kroes, R., Vink, H. H., and Van Esch, G. J. (1966). Short-term toxicity with triphenyltin compounds in rats and guinea pigs. *Food Cosmet. Toxicol.* 4, 35–45.

Verschuuren, H. G., Ruitenberg, E. J., Peetoom, F., Helleman, P. W., and Van Esch, G. J. (1970). Influence of triphenyltin acetate on lymphatic tissue and immune responses in guinea pigs. *Toxicol. Appl. Pharmacol.* 16, 400–410.

Vos, J. G., De Klerk, A., Krajnc, E. I., Kruizinga, W., Van Ommen, B., and Rozing, J. (1984a). Toxicity of bis(tri-*n*-butyltin)oxide in the rat. II. Suppression of thymus-dependent immune responses and of parameters of nonspecific resistance after short-term exposure. *Toxicol. Appl. Pharmacol.* 75, 387–408.

Vos, J. G., Van Logten, M. J., Kreeftenberg, J. G., and Kruizinga, W. (1984b). Effect of triphenyltin hydroxide on the immune system of the rat. *Toxicology* 29, 325–336.

Webber, R. J., Dollins, S. C., Harris, M., and Hough, A. J., Jr. (1985). Effect of alkyltins on rabbit articular and growth-plate chondrocytes in monolayer culture. *J. Toxicol. Environ. Health* 16, 229–242.

Wender, M., Mularek, O., and Piechowski, A. (1974). The effects of triethyltin intoxication at the early stage of extrauterine life on cerebral myelination. *Neuropathol. Pol.* 12, 13–17.

WHO (1980). "Tin and Organotin Compounds. A Preliminary Review." Environmental Health Criteria 15. WHO, Geneva.

Wilkinson, R. R. (1984). Technoeconomic and environmental assessment of industrial organotin compounds. *Neurotoxicology* 5, 141–158.

Wilson, W. E., Hudson, P. M., Kanamatsu, T., Walsh, T. J., Tilson, H. A., Hong, J. S., Marenpot, R. R., and Thompson, M. (1986). Trimethyltin-induced alterations in brain amino acids, amines and amine metabolites: Relationship to hyperammonemia. *Neurotoxicology* 7, 63–74.

Winek, C. L., Marks, M. J., Shanor, S. P., and Davis, E. R. (1978). Acute and subacute toxicology and safety evaluation of triphenyltin hydroxide (Vancide KS). *Clin. Toxicol.* 13, 281–296.

Zuckerman, J. J., Reisdorf, R. P., Ellis, H. V., and Wilkinson, R. R. (1978). Organotins in biology and the environment. *In* "Organometals and Organometalloids: Occurrence and Fate in the Environment" (F. B. Brinckman and J. M. Bellama, Eds.), pp. 388–424. Am. Chem. Soc., Washington, DC.