# TOXICOLOGY: ORGANIC[1]

By Horace W. Gerarde

*Medical Research Division, Esso Research & Engineering Co., Linden, N. J.*
*Bureau of Biological Research, Rutgers—The State University, New Brunswick, N. J.*



## THALIDOMIDE

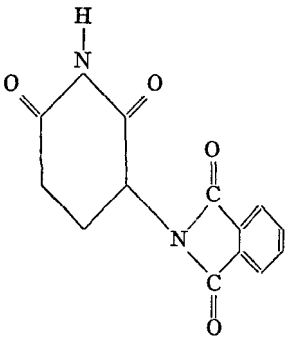
The drug thalidomide will probably go down in history as the greatest therapeutic disaster of all time. The thalidomide incident has had a tremendous impact on toxicology, particularly experimental toxicology which is concerned with proving the safety of drugs by animal experimentation prior to their use in human subjects. Mellen & Katzenstein have written a thorough review covering the thalidomide literature up to late 1962 (1).

*Chemistry.*—The chemical formula for thalidomide (α-phthalimidoglutarimide; 3-phthalimidoglutarimide; 2,6-dioxo-3-phthalimidopiperidine; N-(2,6-dioxo-3-piperidyl) phthalimide; N-phthalyolglutamide) is shown in Figure 1.

This is a white, crystalline, odorless and tasteless compound with a melting point of 271° C. It is insoluble in ether and benzene. Thalidomide has a low solubility in water, methanol, ethanol, acetone and glacial acetic acid but is readily soluble in dioxane, dimethyl formamide, pyridine and chloroform (2). It is only slightly soluble in acids. Thalidomide (2,6-dioxo-3-phthalimidopiperidine) is structurally related to bemegride (Megimide), glutethimide (Doriden), and phenobarbital. See Figure 2.

Thalidomide was first synthesized by Chemie Grunenthal G.m.b.H., Stolberg, West Germany in 1953 (2, 3, 4, 5). It was marketed in West Germany in 1956 (5, 6) under the name of Contergan, in England under the

Fig. 1




Chemical structure of thalidomide

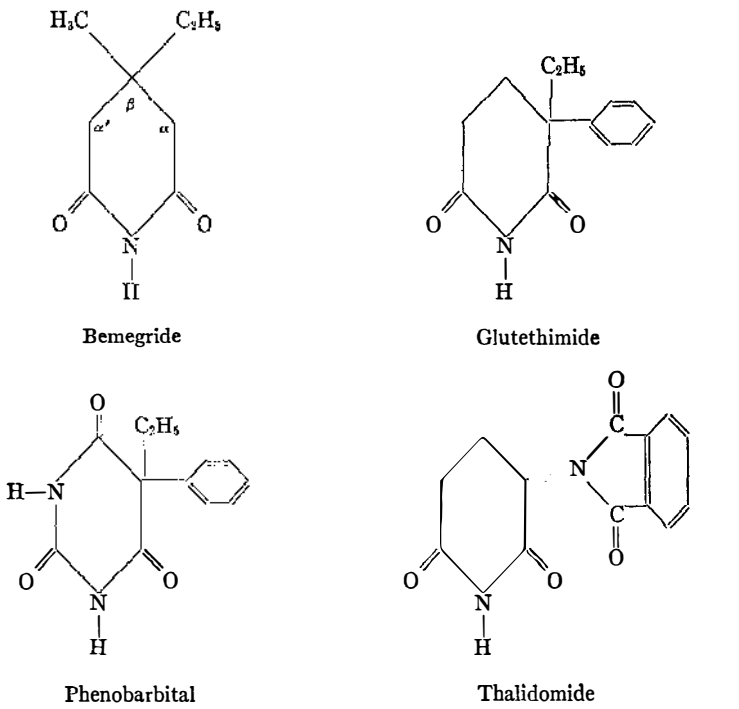
[1] The survey of literature pertaining to this review was concluded July 1, 1963.

name of Distaval (7, 8, 9) and distributed in the United States for experimental purposes and marketed in Canada as Kevadon (3, 10, 11, 12, 13). The A.M.A.-U.S. Pharmacopaeia Nomenclature Committee formally adopted thalidomide as the nonproprietary name of the drug in November 1961 (14).

A survey of foreign scientific journals revealed that thalidomide is known in various areas of the world by the trade names shown in Table 1 (14).

*Pharmacology and toxicology.*—Thalidomide was found to be a highly

FIG. 2




Structural chemical relationships of Bemegride, Glutethimide, Phenobarbital, and Thalidomide

effective sedative-hypnotic acting promptly to give a deep, "natural" all-night sleep without producing a "hangover." The oral dose for sedation is 12.5 to 25 mg and 50 to 200 mg for hypnosis (15). In 1960 it became the favorite sleeping tablet in West Germany available at low cost without a prescription, and was widely used in homes, hospitals and mental institutions.

The toxicity of thalidomide is so low that an oral $LD_{50}$ could not be established in animals (16). No toxic effects were observed in mice dosed with 5000 mg per kg and in dogs after 650 mg per kg. Rats, guinea pigs and rabbits showed no effects on heart rate, blood pressure, respiration, or basal metabolic rate after dosing with thalidomide. The blood picture, serum glutamic

oxalacetic transaminase and urinalysis were normal in rats dosed with thalidomide for twenty-five weeks. These animals had no evidence of neurologic abnormality as determined by function tests and macroscopic and microscopic examination of tissues (1). Somers (2) found that histologic sections of the thyroid glands of thalidomide-treated rats showed only a slight reduction in the colloid material suggesting a mild depression of secretory activity.

Thalidomide appeared to be as "safe" for humans as for animals. Would-be suicides who tried it, after it came on the market, survived large doses without apparent harm (17). An overdose of thalidomide did not induce depression of respiration and heart action, which practically eliminated the possibility of accidental death and suicide through its use. Clinical reports

TABLE 1

TRADE NAMES OF DRUGS CONTAINING THALIDOMIDE

| | |
|---|---|
| N-phthalyol-glutamine | Lulamin |
| Phthalylglutaminsaureimid | Neosedyn |
| α(N-phthalimido) glutarimide | Neurodyn |
| thalidomide | Neurosedyn |
| talidomid | Polygripan |
| thalidomidum | Sedalis |
| Algosediv | Softenon |
| Asmaval | Talimol |
| Contergan | Telargan |
| Distaral | Tensival |
| Distaval | Thalariton |
| Grippex | Thalin |
| Isomin | Thalinette |
| K 17 | Valgis |
| Kedavon | Valgraine |

(*A.M.A. News*, p. 10, Aug. 20, 1962.)

have been published on 17 persons who survived following ingestion of excessive amounts of the drug. One intended suicide ingested 144 times the usual dose and survived. No deaths from overdosages are known (16). Toxic reactions have been reported when thalidomide was taken in doses up to 2.8 gm daily with chlorpromazine (18). These were mainly of the allergic type with an occasional case of jaundice occurring. Glossitis developed in about 25 per cent of these patients after a few days of therapy. This was manifested by rapid denuding of the mucous membrane which in some cases involved the fauces and buccal mucosa. The glossitis was not observed with chlorpromazine therapy alone and it cleared after withdrawal of thalidomide.

A 63 year old woman developed marked myxedema after taking 100 mg of thalidomide daily for two and a half months (19). Since this report which was the first to appear, a number of cases of myxedema following use of thalido-

mide have been published (20 to 23). Disturbances in glucose tolerance following the administration of thalidomide have also been reported (24).

Polyneuritis in four patients taking thalidomide was first reported by Florence (25) in December 1960. The numerous reports which have appeared subsequently definitely establish neuropathy as a toxic effect of thalidomide (26 to 34). The neuropathy has a stocking-glove distribution manifested as sensory changes appearing first in the feet and later in the hands. Some of the terms used to describe the neuropathy are: hyperalgia, hypalgia, muscle pain and tenderness, numbness, "pins and needles," burning, tightness, thickness and "sand in the skin." Motor effects appear later than sensory changes. These occur mainly as weakness, palsy of the dorsiflexors and plantarflexors of the feet, ataxia, and cerebellar symptoms (6, 27, 28, 31, 34). Nerve conduction tests show abnormalities in motor-nerve velocity and sensory-nerve potential (31, 34). Muscle biopsies in two patients reveal changes characteristic of denervation (31). Thalidomide neuropathy is believed to involve the dorsal spinal tracts in addition to peripheral neuritis. Although there is no progression of the disease after thalidomide is discontinued, no one has reported a complete restoration of normal function (1).

In October 1960, two cases of an apparently new syndrome comprising aplasia of the thumbs, the radii and tibiae, stenosis of the duodenum, and capillary hemangioma of the upper lip were shown at the scientific exhibition of the meeting of the German Pediatric Association (17, 35). By 1961 it became quite evident in many places in West Germany that there was an epidemic outbreak of numerous similar cases. Wiedemann (36) was the first to publish an alarming report in which he mentioned 95 cases in a rather restricted geographical region in Western Germany. It appears that gradations of the defect and the biological limits of the syndrome are wider than at first suspected. In the most severe cases there is an anophthalmia and total absence of the arms and legs. These cases are but one extreme of a continuous spectrum, at the other end of which are cases in which only the thumbs are rudimentary or display triphalangism.

The fetal malformations produced by thalidomide are specific and unique according to Dr. W. Lenz, the West German physician who first suspected thalidomide as a possible teratogenic agent (35, 37). The deformity of the limbs known as phocomelia, from the Greek PHOKE, meaning seal, and MELOS, meaning limb, is one of the manifestations of the syndrome which is best described by the term thalidomide embryopathy.

*Metabolism.*—In an experiment with $C^{14}$ tagged thalidomide dosed to rats the $C^{14}$ was not concentrated in any specific organ system. The total excretion of the labeled molecule after 24 hours was 68 per cent in the urine and 10.5 per cent in the feces. After 96 hours 75 per cent appeared in the urine and 19.5 per cent in the feces. A similar experiment conducted in another laboratory revealed that 30 to 40 per cent of the tagged thalidomide appeared in the urine in 48 hours; the remaining radioactive label was found in the feces (1).

Williams (38) has found at least 15 metabolites of thalidomide in urine

from rabbits. Twelve of these have been identified, and most of them have been isolated in crystalline form by column chromatography on alumina and solvent extraction. The compounds are listed in Table 2 (38). The twelve compounds are simple hydrolysis products of thalidomide. The detection of all of them in urine indicates that thalidomide is unstable at physiological pH values. Thalidomide gradually decomposes at 37° C and pH 7.4 in phosphate buffer. Measurements of the rate of decomposition show that 8 per cent had decomposed in 1 hour and 80 per cent in 24 hours. All 12 products were found in paper chromatography. It is clear therefore that thalidomide decomposes spontaneously in the body and that a dose of the drug is in effect equivalent to giving a mixture of 13 compounds, the amounts of which depends on the pH encountered. Thalidomide is stable from pH 2–6 but above pH 6.5 it decomposes spontaneously at room temperature.

In addition to the metabolites shown in Table 2, Williams (38) isolated 3 crystalline fractions which are hydroxylated as shown by infra-red spectra. Unchanged thalidomide was also isolated from the urine. From rabbit feces

TABLE 2

METABOLITES OF DL-THALIDOMIDE IDENTIFIED IN URINE OF RABBITS

| | |
|---|---|
| 1. α-(N-phthaloyliso)-glutamine | 7. α-(*o*-carboxybenzoyl)-glutamic acid |
| 2. α-(N-phthaloyl)-glutamine | 8. α-amino glutarimide |
| 3. α-(N-phthaloyl)-glutamic acid | 9. Phthalic acid |
| 4. α-(*o*-carboxybenzoyl)amino-glutarimide | 10. Glutamine |
| 5. α-(*o*-carboxybenzoyl)iso-glutamine | 11. Isoglutamine |
| 6. α-(*o*-carboxybenzoyl)-glutamine | 12. Glutamic acid |

(Williams, R. T., *Lancet*, **I**, 723, 1963.)

thalidomide and α(*o*-carboxybenzoyl) aminoglutarimide (No. 4 in Table 2) were isolated in crystalline form. The three hydroxy compounds have not yet been identified, but it must be borne in mind that 26 phenolic hydroxy compounds are theoretically possible, not taking into account dextro- and laevo-forms.

If one assumes that the only reactions of thalidomide in the body are hydrolysis of amide linkages and hydroxylation of the benzene ring, and takes into account the fact that the drug itself is a DL-form, more than 100 compounds are theoretically possible.

Compound No. 7 in Table 2, α(*o*-carboxybenzoyl) glutamic acid was found to be a powerful inhibitor of glutamine synthetase.

## TERATOGENIC AGENTS

The identification of thalidomide as a human teratogen and the tragic consequences of its use in pregnant women have intensified the search for other teratogens. Since it was synthesized in 1954 and marketed in 1956 in West Germany, thalidomide has produced anomalies in more than 5000 infants. Projected estimates approach 7000 (39).

Although many agents have been suspected, there are seven definitely known human teratogens: (*a*) Rubella virus, (*b*) the parasite Toxoplasma gondii, (*c*) ionizing radiation, (*d*) carbon monoxide, (*e*) folic acid antagonists, (*f*) some synthetic progestins, and (*g*) thalidomide.

Some of the numerous agents which have been shown to be teratogenic in experimental animals are shown in Table 3 (40). It comes as a shock to learn that the long list of substances found to be teratogenic in the laboratory includes such familiar drugs as salicylates, thyroxine, caffeine, and vitamin A. Even greater surprise may be evoked by the fact that, since 1918, the Nobel Prize for Medicine has, no less than four times, been awarded for the introduction of drugs which have subsequently been shown to produce malformations in laboratory animals. Last year Commissioner Larrick declared to the U. S. Senate Subcommittee on Reorganization and International Organizations: "If you give it to rabbits, and the rabbits have malformed offspring, I think you could say we will not permit the drug on the market." Thus it seems insulin, penicillin, streptomycin, and cortisone might have been proscribed if they had not had the opportunity to prove their therapeutic potential before the thalidomide disaster (41).



## ORGANOMETALLIC COMPOUNDS

*Definition.*—Most workers in the field today consider organometallic compounds as those having direct metal to carbon chemical linkage, not involving intermediate atoms such as oxygen, nitrogen, sulfur, etc. This definition excludes—among other borderline compounds—chelate compounds, salts of organic acids and metal esters as alkoxides. Some experts include organic compounds containing "metalloids" such as boron, silicon, and arsenic. Others include molecules with phosphorus to carbon bonds. In some quarters, "metal/organic compounds" is preferred to avoid confusion with still another more general term "metallo-organics" which broadly embraces metal linkage with atoms other than carbon in organic compounds.

In this survey, organometallics include organic compounds having both covalent and ionic metal to carbon or metalloid to carbon bonds (70).

*Industrial importance.*—Scores of new uses promise wide markets for entirely new compounds made of—or through—organometallics. Grignard reagents, organomagnesium halides first discovered in 1900 are important in the synthesis of drugs such as cortisone, methyltestosterone, vitamin A and atropine and in the manufacture of silicone resins. In volume and value tetraethyllead (TEL) used as an anti-knock agent is the most important organometallic. The annual market for TEL is about a half-billion pounds. Organometallics have played a vital role as catalysts in the impressive advances in plastics and resins since World War II. Foremost of these polymerization catalysts are the light-metal alkyls of the Ziegler-Natta type, used mostly for controlling "linearity" or tactic character of polyolefins. The unique biocidal properties of the trialkyltins have resulted in many uses of organotin compounds as wood preservatives, mildew-proofing, textile preservatives and insecticides (71). Alkylboranes are useful chiefly as pyrophorics in aircraft

applications, as jet fuel igniters to help prevent flame-out of jet engines. Organoboron compounds are important in making "zip" fuels—high-energy aircraft fuels.

TABLE 3

CAUSES OF LIMB MALFORMATION IN EXPERIMENTAL ANIMALS

| Teratogenic Agent | Animal | Deformities | Reference |
|---|---|---|---|
| *Vitamin deficiencies:* | | | |
| Vitamin A | Pigs | Hind-limb defects | Hale (42) |
| Riboflavin | Rats | Aplasia tibia, fibula, radius, ulna. Syndactyly | Warkany & Nelson (43) |
| Pantothenic acid | Rats | Ectrodactyly | Giroud et al. (44) |
| Folic acid | Rats | Syndactyly. Clubfoot | Nelson et al. (45) |
| Vitamin D | Rats | Limb defects | Warkany (46) |
| Vitamin E | Rats | Syndactyly. Clubfoot | Cheng et al. (47) |
| *Vitamin antagonists:* | | | |
| Galactoflavin | Mice | Aplasia tibia, fibula | Kalter & Warkany (48) |
| | Rats | Syndactyly. Clubfoot | Nelson et al. (49) |
| X-methyl folic acid | Rats | Brachydactyly. Syndactyly | Nelson et al. (50) |
| Aminopterin | Rats | Micromelia | Sansone & Zunin (51) |
| X-methyl pantothenic acid | Rats | Clubfoot | Nelson et al. (52) |
| 6-Aminonicotamide | Mice | Aplasia and agensis of limbs | Pinsky & Fraser (53) |
| | Rats | Syndactyly | Murphy (54) |
| Thiadiazole | Rats | Aplasia fibula, ulna. Syndactyly | Murphy (54) |
| *Vitamin excess:* | | | |
| Vitamin A | Mice | Micromelia. Syndactyly | Kalter & Warkany (55) |
| | Rats | Ectromelia | Giroud & Martinet (56) |
| | Rabbits | Syndactyly | Giroud & Martinet (57) |
| *Amino acid antagonists:* | | | |
| Azaserine | Rats | Aplasia long bones. Syndactyly | Murphy (54) |
| CON | Rats | Aplasia long bones | Thiersch (58) |
| *Nucleic acid antagonists:* | | | |
| 2,6-Diaminopurine | Rats | Aplasia limbs | Thiersch (59) |
| 6-Chlorpurine | Rats | Aplasia limbs | Thiersch (59) |
| Thioguanine | Rats | Aplasia limbs | Thiersch (59) |
| 5-Fluorodeoxyuridine | Rats | Syndactyly. Aplasia tibia | Murphy (54) |
| 6-Mercaptopurine | Rats | Syndactyly. Aplasia tibia | Murphy (54) |
| *Alkylating agents:* | | | |
| Chlorambucil ('Leukeran') | Rats | Aplasia long bones. Syndactyly | Murphy et al. (60) |
| Busulphan ('Myleran') | Rats | Aplasia long bones. Syndactyly | Murphy et al. (60) |
| Nitrogen mustard | Rats | Micromelia. Syndactyly | Haskin (61) |
| *Physical agents:* | | | |
| X-radiation | Mice | Oligodactyly. Syndactyly | Murakami et al. (62) |
| | Rats | Syndactyly. Clubfoot | Warkany & Schraffenberger (63) |
| Hypothermia | Hamsters | Adactyly. Syndactyly | Smith (64) |
| *Antibiotics:* | | | |
| Tetracycline | Rats | Micromelia. Syndactyly | Filippi & Mela (65) |
| Penicillin and Streptomycin | Rats | Micromelia. Syndactyly | Filippi & Mela (66) |
| *Miscellaneous:* | | | |
| Caffeine | Mice | Adactyly. Syndactyly | Nishimura & Nakai (67) |
| Salicylates | Rats | Micromelia. Syndactyly | Warkany & Takacs (68) |
| Nicotine | Mice | Adactyly. Brachydactyly | Nishimura & Nakai (69) |

(Millen, J. W., *Lancet*, II, 599, 1962.)

*Triethylaluminum (TEA), $(C_2H_5)_3$ Al, and trimethylaluminum (TMA), $(CH_3)_3$ Al.*—Both TEA and TMA spontaneously ignite on contact with air at room temperature (72, 73). Under controlled conditions small amounts of oxygen react with TEA to form diethylaluminum ethoxide. Further reaction with oxygen leads step-wise to the formulation of ethylaluminum diethoxide and aluminum triethoxide.

Concentrated TEA and TMA react with water to give a violent and sometimes explosive reaction. TEA and TMA also react with alcohols, primary and secondary amines, organic and inorganic acids and even to some extent with paper and cloth to form ethane as the organic by-product (74, 75). Thermal decomposition of TEA yields ethylene and diethylaluminum hydride (76).

The toxicity and the mode of action of TEA and TMA in the animal or human body following their absorption have not been thoroughly investigated. It is not known whether their absorption is or is not a serious problem because these compounds ignite on contact with air or moisture on the skin or other parts of the body including the respiratory tract. The result of this chemical reaction is a burn. The original compound is destroyed in the process and therefore the absorption of a toxic compound is prevented in large part or perhaps entirely.

Contact of the respiratory tract and lungs with vapors of TEA and TMA in sufficient concentration will induce serious injury. The inhalation of the fumes generated by the reaction of such vapor with air may cause moderate to severe illness, depending on the quantities involved and also on the nature of the decomposition products (77).

*Triisobutylaluminum, $Al(iso\text{-}C_4H_9)_3$, and diisobutylaluminum chloride, $AlCl(iso\text{-}C_4H_9)_2$.*—One of the potentially most hazardous reactions of triisobutylaluminum and diisobutylaluminum chloride is oxidation, which is highly exothermic and spontaneous on exposure to air. Since heat is liberated in the reaction a thermal decomposition also occurs forming isobutene which provides an explosion hazard. Perhaps the most violent reaction is that with water; it is instantaneous and explosive with the isobutyl compounds. This type of reaction liberates isobutane and copious quantities of white smoke which is apparently aluminum oxide. The reactions take place by metathesis as follows:

$$Al(iso\text{-}C_4H_9)_3 + 3H_2O \rightarrow Al(OH)_3 + 3\ iso\text{-}C_4H_{10}$$

$$AlCl(iso\text{-}C_4H_9)_2 + 2H_2O \rightarrow Al(OH)_2Cl + 2\ iso\text{-}C_4H_{10}$$

Range finding tests with triisobutylaluminum and diisobutyl chloride indicated no difference between the two compounds. Undiluted materials burned holes in adhesive tape, cloth and polyethylene sheeting in the presence of air. Similarly, holes are burned in the skin wherever the compounds make contact. A 40 per cent dilution in mineral oil burned rabbit skin, but 20, 10, or 5 per cent dilutions caused no reaction. Four grams of a 5 per cent dilution in mineral oil per kilogram of body weight was all rats could ingest with-

out rupture of the stomach. Employees working in a plant using these compounds developed burns which although superficial were painful and very slow in healing. Two of six rats exposed to the fumes from diisobutylaluminum chloride for one hour died the same day of lung hemorrhage. The toxicity of fumes from triisobutylaluminum was somewhat less. The fumes are white and have an unusual musty odor which is detectable even at low concentrations. The animals were in obvious distress which indicates that unconfined humans would be forced from an affected area. In addition to possible lung damage an attack of "fume fever" is possible as a result of inhaling these fumes (75).

*Alkyltin compounds.*—Stoner and co-workers (78) studied the biological effects of a series of mono-, di-, tri-, and tetraalkyltin compounds in rats, rabbits, guinea pigs and fowls. Triethyltin was found to be the most toxic in acute experiments with rabbits. It produced muscular weakness, tremors, convulsions and death at sufficiently high doses. Repeated doses of triethyltin produced mainly muscular weakness. The principal site of action of the alklyltin compounds was the central nervous system. Tin did not appear to be concentrated in any particular area in the brain or spinal cord (78).

A single dose of 50 mg/kg of dibutyltin dichloride fed to rats caused temporary dilatation of the stomach and an inflammatory lesion of the bile ducts. Salts with aliphatic chains less than 3 carbons and more than 6 did not cause the bile duct lesions. Repeated daily doses resulted in a high proportion of deaths within six days as a result of bile duct injury and severe hepatitis. Application of 10 mg/kg to the skin also produced lesions of the bile ducts and skin injury at the site of contact. Intraperitoneal injection of 7.5 mg/kg to rats produced symptoms of shock followed by death. Mice were more susceptible than rats. Dibutyltin chloride was also more toxic to rabbits but guinea pigs tolerated repeated doses of 50–100 mg/kg without any general toxic manifestation or evidence of biliary tract lesions. Rats and rabbits showed signs of toxicity after application of 10 mg/kg to the skin. Guinea pigs were again more resistant by the percutaneous route of administration. The relative intravenous toxicity of the dialkyl dichloro tin salts administered to rats was as follows: The $LD_{50}$ for dimethyl was 40 mg/kg; for diethyl, 25 mg/kg; for di-*n*-propyl, 7 mg/kg; for diisopropyl, 15 mg/kg and for dibutyl, 5 mg/kg. The $LD_{50}$ for di-2-ethylhexyltin dichloride was 5 mg/kg.

In general, the trialkyl compounds produced reactions which differ from those caused by the dialkyltin derivatives. They appear to act mainly on the central nervous system. Toxic manifestations include muscular weakness, tremors, irritability, collapse and death. Edema of the white matter of the central nervous system was the principal finding at autopsy (79).

Lesions of the skin in process workers exposed to dibutyltin chloride were reported by Lyle (80). Injury to the skin mainly from accidental splashes with an alcoholic solution of dibutyltin chloride occurred with increasing frequency about three years after the plant had started manufacture of this compound. The lesion is of the specific primary irritant type. Typically it

consists of an erythema limited to the area of contact which causes moderate itching but is otherwise painless. There is a latent period of about eight hours after initial contact. The lesions heal spontaneously in a few days. Exposure to vapors of the tin compound cause a diffuse type of reaction. The vapors originate from contaminated clothing worn by the worker, who complains that the affected area (ventral surfaces and flexures) feels sticky or that it is rubbed by the clothing.

Transient nausea and cough have occasionally followed inhalation of fumes arising from the manufacturing process but there was no evidence of a systemic toxic effect among the workers.

Elsea & Paynter (81) conducted a toxicological study on bis(tri-*n*-butyltin)oxide [$(C_4H_9)_3$-Sn-O-Sn$(C_4H_9)_3$.] The acute oral $LD_{50}$ for male albino rats was found to be 194 mg/kg and 148 mg/kg when administered in aqueous and oil media respectively. It was fed in the diet of groups of male albino rats at levels of 32, 100 and 320 ppm for 30 days. All test animals exhibited growth suppression increasing in severity with increased feeding levels. Food consumption for the 32 and 100 ppm groups was comparable to that of the control group throughout the study. Food consumption for the 320 ppm group was greatly reduced. All animals in the 32 and 100 ppm groups survived; four animals in the 320 ppm group survived.

The acute dermal $LD_{50}$ of bis(tri-*n*-butyltin) oxide for albino rabbits is in the range of 11.7 g/kg. A single application of the undiluted material produced a moderate degree of dermal irritation and there were gross signs of systemic toxicity from percutaneous absorption. Five daily applications of newsprint-type paper containing 8.0 ppm of the bis(tri-*n*-butyltin) oxide to the skin of rabbits produced no gross evidence of dermal irritation or systemic toxicity.

Bartalini (82) found that daily doses of 100 mg/kg of di-*n*-butyltin oxide caused evidence of systemic toxicity in two or three days and deaths by the fifth day after dosing. Histologic examinations showed diffuse, severe degenerative changes in the liver, glomeruli and renal tubules. With daily doses of 25 mg/kg the rats died within 11 days with the same signs of intoxication and almost identical pathological changes. Chronic toxicity was studied by daily administration of 2.5 mg/kg dibutyltin oxide for 60 days. The rats were sacrificed still in apparent good health. Histological examinations showed slight liver changes and some changes in renal tubules with occasional refractive changes in the tubular epithelium. In general the picture was that of a reversible type of injury.

Alajouanine and co-workers (83) summarized their chemical studies on 210 cases of intoxication resulting from diethyltin diiodide. This was an ingredient of a preparation used for the treatment of furunculosis. More than 100 deaths from cerebral edema were reported in this therapeutic disaster. Some survivors developed paraplegia and other manifestations of central nervous system injury. The following typical case is reported by Lewis (84).

> A 22 year old man ingested a number of capsules about two weeks prior to the onset of severe headache. During the following week he had nausea, vomiting, and

palpitations. He was admitted to the hospital in a confused state and found to have bilateral papilledema and generalized weakness, but no localizing neurological signs. Results of urine and complete blood count examinations were normal. He subsequently developed paraplegia and retention of urine, became comatose, and died three weeks after the onset of his symptoms."

The following evidence presented by Cremer (85, 86), (*a*) inhibition of the metabolism of brain slices, (*b*) increase in water content of the brain and spinal cord, and (*c*) the identification of triethyltin in tissues of animals given tetraethyltin, leaves little doubt that tetraethyltin is converted into triethyltin in vivo by rats and rabbits. Triethyltin has been shown to be the principal conversion product. Since the symptoms of poisoning from a dose of tetraethyltin develop only after an initial latent period, and the increased inhibition of brain slice metabolism can be closely correlated with increased amounts of triethyltin in the brain, it appears that the toxic action of tetraethyltin is due solely to the triethyltin formed from it in the liver. Tetraethyltin added in vitro at a concentration of 0.15 m*M* had no effect on metabolism of brain slices.

The data obtained strongly suggests that the conversion of tetraethyltin is enzymatic. The chemistry of the conversion appears to involve an oxidative mechanism. Aldridge (87) studied the effect of an homologous series of trialkyltins on oxidative phosphorylation. Triethyltin was found to be the most active inhibitor of oxidative phosphorylation. Of the trialkyltins tested only tri-*n*-octyltin was found to be inactive. Inconsistent results were obtained with tri-*n*-hexyltin. The behavior of the high molecular weight homologues is believed to be due to their low water solubility. It is postulated that the mechanism of action of the alkyltins resides in a blocking in the energy trapping and transferring reactions between electron transport and the formation of adenosine triphosphate (ATP).

The results obtained by Stoner & Threlfall (88) on the effect of triethyl tin on incorporation of $P^{32}$ into the nervous system suggest interference with the utilization of chemical energy. Although $P^{32}$ was incorporated into the energy-rich phosphates, its further distribution was impaired. In this action triethyltin resembles sodium pentobarbital and chlorpromazine (89, 90).

*Mercury.*—Organic mercury compounds have been used for a number of years in germicidal agents, and more recently as seed disinfectants and as biocidal agents in anti-fouling paints. These compounds may be solids, or liquids at room temperature. Depending on chemical structure, some of the lower molecular weight alkyl derivatives have a considerable vapor pressure at room temperature and consequently are hazardous materials to handle.

The toxicity of organic mercurials varies over a wide range depending on chemical constitution. The toxic manifestations differ markedly from the effects of inorganic mercury cation which is described as a general protoplasmic poison by virtue of its nonspecific combination with protein. Thirty-nine cases of poisoning summarized by Kurland and co-workers (91) are listed in Table 4. In almost all of these cases contact was believed to be by inhalation rather than ingestion. Note the predominance of neurotoxic effects

## TABLE 4

### SURVEY OF REPORTS OF POISONING WITH ORGANIC MERCURY COMPOUNDS

| Case No. | Author | Preparation | Clinical Course | Type of Work or Other Indication of Source of Exposure |
|---|---|---|---|---|
| Canadian | | | | |
| 1. | Hill (1943) | Diethyl mercury | Stomatitis; salivation; blue gum line; metallic breath; memory loss; depression; irritability; death | Storehouse exposure |
| 2. | Hill (1943) | Diethyl mercury | | Storehouse exposure |
| English | | | | |
| 3. | Edwards (1865) | Dimethyl mercury | Numbness of hands; deafness; poor vision; sore gums; unsteady gait; death in 11 days | Laboratory assistant |
| 4. | Edwards (1865) | Dimethyl mercury | Sore gums; salivation; numbness of feet, hands, tongue; deafness; blindness; ataxia; dysphagia; irritability; death one year later | Laboratory assistant |
| 5. | Edwards (1865) | Dimethyl mercury | No details available | Laboratory assistant |
| 6. | Hunter, Bomford & Russell (1940) | Methyl mercury compounds | Tremor; ataxia, dysarthria; constriction of visual fields. All remained incapacitated | 4 of 16 men exposed in a seed dressing factory were affected. 4 others were asymptomatic and 8 were asymptomatic but excreted mercury in urine |
| 7. | Hunter, Bomford & Russell (1940) | Methyl mercury compounds | | |
| 8. | Hunter, Bomford & Russel (1940) | Methyl mercury compounds | | |
| 9. | Hunter, Bomford & Russell (1940) | Methyl mercury compounds | | |
| German | | | | |
| 10. | Prumers (1870) | Ethyl mercury compound | No details available | Mentions that several chemists had been poisoned |
| 11. | Jansen (1929) | Tillantin R<br>Alkoxyalkyl mercury | Recovery | Careless while treating seed. Sowed by hand on a warm day |
| 12. | Veilchenblau | Abavit B<br>Ethanol mercury carbamide | Recovery | Treated seed without protection. Sowed by hand on a warm day |
| 13. | Koelsch (1937) | Germisan. Phenyl mercury pyrokatekin compound | Recovery—few details available | Careless when treating seed |

TABLE 4—(*continued*)

| Case No. | Author | Preparation | Clinical Course | Type of Work or Other Indication of Source of Exposure |
|---|---|---|---|---|
| 14. | Koelsch (1937) | Ceresan. Phenyl mercury acetate | Recovery—few details available | Careless spreading of disinfectant |
| 15. | Koelsch (1937) | Phenyl mercury acetate | Recovery—few details available | Chemist |
| 16. | Koelsch (1937) | Various organic compounds | Recovery—few details available | Chemist |
| 17. | Koelsch (1937) | Various organic compounds | Recovery—few details available | Chemist |
| 18. | Koelsch (1937) | Various organic compounds | Recovery—few details available | Chemist |
| Swedish | | | | |
| 19. | Herner (1945) | Methyl mercury iodide | Ataxia; dysarthria; astereognosis; narrowing of visual fields; numbness of tongue, lips, and fingers; irritable; memory unstable | Factory worker |
| 20. | Lundgren-Swensson (1948) | Various alkyl compounds | Stomatitis; lethargy; nausea; headache; tremor; recovery | Laboratory assistant |
| 21. | Lundgren-Swensson (1948) | Probably methyl compound | Tenesmus, diarrhea, nausea, complete recovery | Laboratory assistant (careless) |
| 22. | Lundgren-Swensson (1948) | Probably methyl compound | Recovery | Laboratory assistant (careless) |
| 23. | Lundgren-Swensson (1948) | Fibrosan (alkyl mercury) | Numbness in hands and around mouth, ataxia, inability to button clothes, dysarthria, narrowing of visual fields, central hearing defect; death | Sawmill worker |
| 24. | Lundgren-Swensson (1948) | Various alkyl compounds | Anorexia, metallic taste, tremor, emotional lability, normal visual fields; remained incapacitated | Seed controller |
| 25. | Lundgren-Swensson (1948) | Methyl mercury compounds | Emotional lability, headaches, salivation, metallic taste in mouth, tremor, incoordination of fingers; complete recovery | Seed controller |
| 26. | Lundgren-Swensson (1948) | Methyl mercury compounds | Lethargy, headaches, emotional lability, deterioration of memory and mental functions; complete recovery | Laboratory assistant |
| 27. | Lundgren-Swensson (1948) | Alkyl mercury compounds | Headache, tremor of hands, dermatitis of hands, memory loss, irritability; complete recovery | Handled mercury compounds |
| 28. | Ahlmark (1948) | Methyl mercury compounds | Ataxia, dysphagia, diplopia, irritability; death | Seed dressing |
| 29. | Ahlmark (1948) | Methyl mercury | Recovery | Factory packer |

TABLE 4—(*continued*)

| Case No. | Author | Preparation | Clinical Course | Type of Work or Other Indication of Source of Exposure |
|---|---|---|---|---|
| 30. | Ahlmark (1948) | Methyl mercury compounds | Recovery | Factory packer |
| 31. | Ahlborg & Ahlmark (1949) | Methyl mercury compounds | Invalid | Sowing large quantity of disinfected seed by hand |
| 32. | Tejning (1951) | Methyl mercury diayandiamide (Panogen) | Invalid | Ate disinfected seed |
| 33. | Hook, Lundgren & Swensson (1954) | Methyl mercury compounds | Salivation, metallic taste, ataxia, numbness in legs, dysarthria, positive Romberg test, blurred optic discs, comatose; death | Seed dressing plants |
| 34. | Hook, Lundgren & Swensson (1954) | Ethyl mercury compounds | Headache, blurred vision weakness, nausea, tremor, impaired memory, numbness in face and right hand, dysarthria | Farmer (used seed dressing) |
| American 35. | Ian A. Brown (1954) | Phenyl mercury acetate (Cerasan) | Clinical syndrome of amyotrophic lateral sclerosis, death; autopsy confirmed amyotrophic lateral sclerosis; large quantities of mercury found in urine | Farmer |
| 36–39. | Ian A. Brown (1954) | Phenyl mercury acetate (Ceresan) | "Combined motor system involvement"—no other details available | Farmers |

(Kurland, L. T., Faro, S. N., and Siedler, H., *World Neurol.*, 1, 370, 1960.)

in these cases. Some organic mercury compounds, particularly the alkyl derivatives, have a predilection for the brain. Several workers have explained this on the basis that the alkyl mercury compounds readily penetrate the blood-brain barrier. The concentration of mercury in the cerebrospinal fluid is considerably greater in animals receiving small quantities of organic compounds than in those receiving equal amounts of mercury cation (92, 93). Swensson & Lundgren (94) found that organic mercurials are carried mainly in the erythrocytes whereas mercury cation is concentrated mainly in the plasma. Friberg (95) injected a methyl mercury compound and mercuric chloride in rats over a 10 day period. The mercury level was 100 times greater in the blood, twice as high in the liver and 10 times greater in the brain in the animals dosed with methyl mercury compound than in those receiving mercuric chloride. The kidney was the only organ in which the mercury concentration was higher after injection of the inorganic compound.

The concentration of mercury in tissues of individuals who died of mercury poisoning is shown in Table 5.



TABLE 5

RELATIONSHIP OF MERCURY CONTENT IN VARIOUS TISSUES OF PATIENTS WHO DIED OF MERCURY POISONING

| Author, Compound, and Duration of Symptoms (if reported) | Liver (ppm) | Kidney (ppm) | Brain (ppm) |
|---|---|---|---|
| *Inorganic Mercury* | | | |
| Lomholt | | | |
| 50-year-old man, died 3 days after ingestion of mercuric chloride | 32 | 70 | 2 |
| Lomholt | | | |
| 18-year-old woman, died 6 days after ingestion of mercuric chloride | 3 | 16 | 1 |
| Ludwig & Zillner | | | |
| mercuric chloride | 68 | 144 | 1 |
| Ludwig & Zillner | | | |
| 32-year-old man, died 7 days after drinking 10 g mercuric chloride | 30 | 28 | 0.2 |
| *Organic Mercury* | | | |
| Lundgren-Swensson | | | |
| | | | 10 (basal ganglion) |
| | | | 5 (cerebellum) |
| Alkyl mercury inhalation for 5 years, died 1 month after onset of symptoms | 14 | 3 | 5 (corpus callosum) 3 (pons) |
| Hook, Lundgren & Swensson | | | |
| Methyl mercury inhalation for 3 years, died 2 months after onset of symptoms | 39 | 27 | 12 |
| *Minamata Disease Cases* | | | |
| 26 days | 38 | 48 | 15 |
| 90 days | 36 | 29 | 5 |
| 553 days | 26 | 37 | 5 |
| Control case—Minamata patient dying from other control cause | 1 | 3 | 0.1 |

(Kurland, L. T., Faro, S. N., and Siedler, H., *World Neurol.*, 1, 370 1960.)

*Minamata disease.*—In 1953, a severe neurologic abnormality was recognized in persons living in the vicinity of Minamata Bay, Japan. Eighty-three cases have been reported most of which have been fatal or suffered permanent severe disability. Mercury-contaminated effluent from a large chemical manufacturing plant which emptied into the bay has been shown to be the source of the toxic material which accumulated in the tissues of fish and shellfish in the bay area. Although the bay is not suitable for commercial fishing, it has been used regularly as a source of seafood for many of the families inhabiting eleven small villages along the shores of the bay.

Minamata disease may be acute or subacute. Usually it begins with a progressive numbness of the distal parts of the extremities and often of the lips and tongue. This is followed by an ataxic gait, clumsy use of the hands, dysarthria, dysphagia, deafness, and blurring of vision. Spasticity and rigidity are also often present, although muscle atrophy is rarely seen. Insomnia, agitation, and loss of emotional control are often found. Generalized convulsions, stupor and coma have been reported in a few cases. Most patients have abnormal involuntary movements, such as choreoathetosis, myoclonus, and coarse resting and action tremors. In severely affected patients intellectual impairment results. Children appear to be particularly susceptible to having serious residual effects. The immediate causes of death are aspiration pneumonitis, intercurrent infection, and inanition. The frequency of the signs and symptoms in Minamata disease is shown in Table 6. The duration of the disease in fatal cases ranges from 26 days to 4 years. The following is a typical case described by Kurland and co-workers (91).

> A 14-year-old boy who had been agile and bright before his illness is said to have eaten a large number of crabs and small fish from a posted area of Minamata Bay during a 10-day period in July, 1958. A few weeks later he noted numbness around his mouth and in his hands and feet. He did not have fever, headache, or a stiff neck. He was dysarthric and became clumsy in buttoning his clothes and handling his chopsticks; his family observed that he staggered slightly when walking. His auditory acuity and attention span diminished, and he developed the mannerisms and behavior of a younger child. He was hospitalized, and when he was examined three weeks after the onset of symptoms, salivation, behavior inappropriate for his age, clumsiness of his hands, a slightly ataxic gait, hearing loss, and dysarthria were noted. On confrontation there was suggestive constriction of his peripheral fields of vision; fundoscopic examination revealed no abnormality; his reaction to light, his accommodation, and his eye movements were normal; there was no nystagmus. No muscle weakness was noted, and the sensory examination was essentially normal.
>
> The boy displayed a slight action tremor, more pronounced on the left side with finger-to-nose testing and more on the right side in heel-to-shin testing. His reflexes were active and equal except for those of the right lower extremity, which were slightly more active than the left. The plantar response was flexor on the left and extensor on the right. His memory for most recent events was adequate, but he was unable to perform calculations beyond the 8- or 9-year-old level. When observed in 1960, he was still hospitalized in the Minamata City Hospital. Aside from some improvement in learning, the clinical picture was essentially unchanged, and little effort was being made to rehabilitate him for a life outside the hospital.

TABLE 6

FREQUENCY OF SIGNS AND SYMPTOMS IN 24 PATIENTS WITH MINAMATA DISEASE

| Symptom or Sign | No. | Per Cent |
|---|---|---|
| Paresthesia | 24 | 100 |
| Constriction of visual fields | 17* | 95 |
| Hearing loss | 20 | 83 |
| Speech disorders | 20 | 83 |
| Psychologic disturbances | 18 | 75 |
| Excessive salivation | 7 | 29 |
| Excessive sweating | 6 | 25 |
| Dysdiadochokinesia | 22 | 92 |
| Disordered handwriting | 22 | 92 |
| Unsteady gait | 21 | 88 |
| Intention tremor | 19 | 79 |
| Positive Romberg sign | 13 | 54 |
| Rigidity | 4 | 17 |
| Chorea | 3 | 13 |
| Ballismus | 3 | 13 |
| Athetosis | 2 | 8 |
| Exaggerated muscle stretch reflexes | 8 | 33 |
| Reduced muscle stretch reflexes | 2 | 8 |
| Pathologic reflexes | 3 | 13 |

* Of 18 patients whose peripheral visual fields were examined.
(Kurland, L. T., Faro, S. N., and Siedler, H., *World Neurol.*, 1, 370 1960.)

His physical disability was considered mild, but his inability to calculate and remember complex Japanese written characters made it impossible for him to continue in school.

The pathologic changes are confined primarily to the central nervous system. Grossly there are found varying degrees of cerebral edema, cerebellar atrophy, atrophy of the calcarine, pre- and postcentral cerebral cortex and punctate hemorrhages. The cerebellar hemispheres show diffuse cellular degeneration with gliosis microscopically. The cortical areas, the hypothalmus, midbrain and basal ganglia are also affected. The optic nerves, cranial nerve roots, sympathetic ganglia, anterior and posterior roots and peripheral nerves show no involvement. The spinal cord shows only minor changes with preservation of the anterior horn cells, although there is dilatation of the perivascular spaces with softening. There is no true demyelination.

A similar histopathology of the central nervous system was found in cats, crows, sea birds, and fishes succumbing spontaneously to Minamata Disease as well as for experimental animals fed contaminated fish and shellfish from Minamata Bay (91).

A striking similarity to Minamata Disease is apparent in clinical reports of workers suffering from organic mercury intoxication from industrial

sources. Animals fed dimethyl mercury and ethyl mercuric chloride have clinical and pathological effects similar to that produced by seafood taken from Minamata Bay.

The mercury compound in the seafood is not extractable with organic acid or basic solvents. There is a possibility that the compound is bound to protein as a complex which is released by digestive enzymes.

Uchinda and co-workers (96, 97, 98) have conducted extensive tests in attempting to isolate and characterize the causative agent of Minamata Disease. An organic mercury compound has been isolated in crystalline form from toxic shellfish. Analytical data indicate that the compound is methyl-methyl mercuric sulfide, $CH_3$-Hg-S-$CH_3$.

The typical symptoms and signs of Minamata Disease were observed in animals fed a synthetic sample of methyl-methyl mercuric sulfide (97).

*Tri- and tetraalkyllead.*—In general, the alkyl derivatives of lead are highly toxic compounds which are absorbed through the skin, the mucosa of the alimentary tract and the alveoli. Some of the physical properties of tetraethyl and tetramethyllead are shown in Table 7 (99).

These compounds are probably distributed in nonionic form and being lipid soluble are concentrated in the brain, body fat and liver. Because of this selective distribution, manifestations of poisoning are dominated by involvement of the central nervous system and differ from those of inorganic lead poisoning.

TABLE 7

PROPERTIES OF TETRAETHYLLEAD AND TETRAMETHYLLEAD

| Physical Properties | Tetraethyllead (Neat) | Tetramethyllead (Neat) |
|---|---|---|
| Physical form | Watery white oily liquid | Watery white oily liquid |
| Chemical formula | $(C_2H_5)_4Pb$ | $(CH_3)_4Pb$ |
| Odor | Faint, fruity | Faint, fruity (probably odorless in chem. pure state) |
| Saturated liquid density at 20 C | 1.65 g/ml | 1.99 g/ml |
| Vapor pressure at 20 C | 0.27 mm Hg | 22.5 mm Hg |
| Boiling point* | 199 C | 110 C |
| Freezing point | −130.2 C | −30.3 C |
| Flash point (open cup)* | 85 C | About 38 C |
| Viscosity at 20 C | 0.87 cps | 0.53 cps |
| Refractive index $N^{20}/D$ | 1.520 | 1.512 |
| Solubility in water at 22 C | 0.18 ppm | 18.0 ppm |
| Solubility in gasoline | Soluble in all proportions | Soluble in all proportions |

* Neither of these compounds should be heated, since they tend to decompose rapidly, even violently, at sufficiently elevated temperatures. The commercial products contain stabilizers and should not be distilled.

(Davis, R. K., Horton, A. W., Larson, E. E., and Stemmer, K. L., *Arch. Environ. Health*, **6**, 473, 1963.)

Tetraethyllead (TEL), which has been used for many years as an anti-knock gasoline additive, is still the most important industrial organo-lead compound. The principal manifestations of exposure to tetraethyllead are insomnia, asthenia, tremors, neuromuscular pain, hallucinations, mania, delusions and frank psychosis. Because of the large dilution factor, 3–4 ml per gallon of gasoline, the normal use of "leaded" gasoline does not present a lead intoxication hazard. When TEL was first introduced in 1923 Public Health authorities were apprehensive about hazards which its exhaust products might create. Extensive studies in the U. S. and abroad have shown that there is no need for concern at normally used concentrations of TEL, even under the most adverse conditions such as in tunnels, heavy traffic and confined garages. The ventilation required to prevent excessive concentrations of exhaust gases and carbon monoxide provide ample dilution.

Analyses of air in many cities have shown lead concentrations, as judged by current hygienic criteria, to be insignificant. The data suggest that much of the lead burned in gasoline is not exhausted in forms which can remain suspended in the atmosphere. Approximately 20 to 30 per cent of lead burned in fuel is retained in passenger car exhaust system deposits and lubricating oil. The balance, 70 to 80 per cent is exhausted over 20,000–30,000 mile periods of city and country driving. The lead is exhausted principally as $PbCl \cdot Br$, $NH_4Cl \cdot 2PbCl \cdot Br$ and $3Pb_3(PO_4)_2$ (100).

The intraperitoneal injection of trimethyllead induces in rats signs of central nervous system toxicity at low doses (tremors and convulsions). The $LD_{50}$ by this route is 25.5 mg/kg. Tetramethyllead is less toxic than trimethyllead. A dose of 34 mg/kg given intravenously to rats caused no signs of poisoning. Large oral doses of tetramethyllead (250 to 500 mg/kg) fed to rats produced signs of intoxication identical with those elicited by trimethyllead. An immediate toxic reaction was observed in rabbits dosed intraperitoneally with 7.5 and 15 mg/kg of trimethyllead. The intravenous dosing of 20 and 40 mg of tetramethyllead did not produce toxic manifestations in the rabbit. According to Davis and co-workers (99)

> The first detectable sign of the toxic effect of the absorption of either compound was that of an irritable behavior. The rats became difficult to handle. After an initial period of this behavior they became uncoordinated for a time and then developed a state of seeming combativeness; rising on their hind legs, as though buffeting each other with eyes partially closed, they bumped into each other or into the wall of the cage, sometimes biting each other, seemingly without purpose. After periods of relative calm, they could be stimulated by noise or mechanical means to begin the cycle again, or their activity would begin again without apparent external stimulus. Animals further exposed after the development of these signs developed convulsions, after which the animal collapsed for a time. When exposure was discontinued before or early in the convulsive stage, the rats usually recovered completely in three to four weeks. Otherwise, the intoxication progressed, and the animals died in coma.
>
> The signs of intoxication in the dogs differed from those in the rats. Tremors and muscle twitching developed gradually, first in the extremities and then in the trunk. The extremities moved continuously when the animals lay down. Replaced

TABLE 8

COMPARATIVE MORTALITY OF RATS FOLLOWING INHALATION OF TETRAMETHYLLEAD OR TETRAETHYLLEAD, AND LEAD CONTENT OF THEIR TISSUES

| Compound | Concent. of Compound in Air Breathed (Mg/m³) | Duration of Expos. (Hours) | Mortality (Died/Exposed) M | F | Total | Aver. Interval Between Last Period of Exposure and Death (Days) | No. Pooled for Chem. Anal. | Aver. Concent. of Pb in Major Viscera* (Mg/100 G) | Aver. Concent. of Pb in Urine (Mg/Liter) |
|---|---|---|---|---|---|---|---|---|---|
| Tetramethyllead | 63.0 | 10×7 | 5/5 | 4/5 | 9/10 | 2 | 5 | 10.12 | — |
| | 49.0 | 18×7 | 5/5 | 4/5 | 9/10 | 0.25 | 8 | 10.06 | 8.38 |
| | 22.0 | 35×7 | 5/5 | 3/5 | 8/10 | 2 | 8 | 7.54 | 4.53 |
| | 12.0 | 150×7 | 4/5 | 0/5 | 4/10 | 1† | 4 | 5.47 | |
| | | | | | | 0.50† | 3 | 2.25 | 1.94 |
| | | | | | | 40† | 3 | 0.67 | |
| Tetraethyllead | 46.0 | 5×7 | 3/5 | 5/5 | 8/10 | 3 | 8 | 1.99 | — |
| | 22.0 | 14×7 | 4/5 | 5/5 | 9/10 | 0.25 | 9 | 2.99 | — |
| | 12.0 | 150×7 | 0/5 | 0/5 | 0/10 | 1‡ | 4 | 0.78 | 5.22 |
| | | | | | | 39‡ | 5 | 0.43 | |
| Control | 0 | 150×7 | 0/10 | 0/10 | 0/20 | 12 | 20 | 0.12 | 0.39 |

* Viscera included: lung, liver, kidney, spleen, heart, and brain.

† Of the ten rats that inhaled 12 mg per cubic meter, four died after 116 periods. These fatalities were complicated by intercurrent infections. Three of the remainder were killed one-half day after the 150th period, and the remaining three were killed 40 days later.

‡ Killed for further examination.

(Davis, R. K., Horton, A. W., Larson, E. E., and Stemmer, K. L., *Arch. Environ. Health*, **6**, 473, 1963.)

on their feet, they stood on stiff, wide-spread legs. At the peak of the hyperactivity the clinical picture was similar to that of chorea. Later after continued exposure, convulsions ensued, followed by death in coma.

The comparative mortality of rats and dogs following inhalation of tetramethyllead or tetraethyllead is shown in Tables 8 and 9.

The intraperitoneal $LD_{50}$ for triethyllead in rats is 11.2 mg/kg. The intravenous $LD_{50}$ in rats for tetraethyllead is 15.4 mg/kg.

*Distribution in tissues and metabolism.*—Considerable amounts of triethyllead were found in the tissues of rats after intraperitoneal dosing with tetraethyllead. The distribution of the triethyllead in the tissues of animals dosed with tetraethyllead was virtually identical with that found after injection of triethyllead. Only small amounts of trimethyllead were found in the tissues of rats dosed with tetramethyllead. The lead content of the tissues of rats and dogs following inhalation of TML and TEL is shown in Tables 8 and 9.

The conversion in vivo of tetraalkyllead to trialkyllead is now well-established (101 to 104). Tetraethyllead is converted rapidly to the more toxic trialkyl derivative. The conversion of tetramethyllead to trimethyllead is much slower. The toxicity of the tetraalkyl compounds apparently depends on the rate of conversion to the more toxic trialkyl derivative.

TABLE 9

COMPARATIVE MORTALITY OF DOGS FOLLOWING INHALATION OF TETRAMETHYLLEAD OR TETRAETHYLLEAD, AND LEAD CONTENT OF THEIR TISSUES

| Compound | Init. Body Wt. (Kg) | Concent. of Compound in Air Breathed ($Mg/m^3$) | Duration Expos. Before Fatal Outcome (Hours) | Aver. Interval Between Last Period of Exposure and Death (Days) | Aver. Concent. of Pb in Major Viscera* (Mg/100 G) | Aver. Concent. of Pb in Urine (Mg/Liter) | Aver. Concent. of Pb in Blood (Mg/100 G) |
|---|---|---|---|---|---|---|---|
| Tetramethyllead | 10.8 | 44.0 | 8×7† | 1 | 1.03 | 4.02 | 0.13 |
| | 9.0 | 23.0 | 9×7 | 0 | 0.85 | 4.52 | 0.05 |
| | 5.4 | 12.0 | 15×7 | 1 | 0.69 | | |
| | 11.0 | 12.0 | 14×7 | 2 | 0.74 | 0.64 | 0.04 |
| | 11.6 | 4.0 | 107×7 | 0 | 0.79 | | |
| | 11.0 | 4.0 | 84×7 | 0 | — | 0.59 | 0.06 |
| Tetraethyllead | 10.6 | 42.0 | 7×7† | 0.25 | 1.22 | 10.20 | 0.12 |
| | 13.2 | 22.0 | 30×7 | 0 | 2.96 | 2.29 | 0.14 |
| | 5.9 | 12.0 | 29×7 | 3 | 1.02 | | |
| | 10.2 | 12.0 | 24×7 | 0 | 0.67 | 2.42 | 0.06 |
| Control | 6.8 | 0 | >130×7 | — | 0.05 | 0.16 | 0.02 |

* Viscera included: lung, liver, kidney, spleen, heart, and brain.

† Each line represents the period of survival of a single dog subjected to exposure to an alkyllead compound. (Two control dogs were killed at the end of the period of time indicated.)

(Davis, R. K., Horton, A. W., Larson, E. E., and Stemmer, K. L., *Arch. Environ. Health*, 6, 473, 1963.)

Trimethyl and triethyllead decrease oxygen consumption when added to rat brain and cortex slices in vitro. Tetramethyl and tetraethyllead did not have this effect except at much higher concentrations. A decreased in vitro oxygen consumption was also found in brain slices taken from rats previously exposed to triethyl and tetraethyllead.

*Cobalt hydrocarbonyl.*—Cobalt is used as a catalyst in the industrial synthesis of alcohols known as the oxo process. Cobalt carbonyls are formed in the process by the reaction of synthesis gas (carbon monoxide and hydrogen) with the cobalt catalyst. Although the industrial operations are carried out in closed systems there is a possibility of exposure to low concentrations during normal operations and to higher levels in case of an accident.

There are three known cobalt carbonyls: cobalt hydrocarbonyl, $H \cdot Co(CO)_4$, a gas at room temperature; dicobalt octacarbonyl, $[Co(CO)_4]_2$ a solid with a vapor pressure of about 0.1 mm Hg at room temperature; and tetracobalt dodecacarbonyl, a solid having a very low vapor pressure at room temperature.

Cobalt hydrocarbonyl decomposes rapidly in air to inorganic cobalt compounds which are believed to be cobalt carbonate or one of the hydrated oxides. Palmes and co-workers (105) found that the $LC_{50}$ for rats for a single 30 minute exposure was approximately 165 mg $Co/m^3$. The acute clinical signs of intoxication and the gross and microscopic pathology were indicative of chemical pneumonitis. There was a marked similarity between the acute effects of cobalt hydrocarbonyl and nickel carbonyl. On the basis of 30-minute rat $LC_{50}$'s the cobalt compound is considered to be about one-half as potent as nickel carbonyl $Ni(CO)_4$. The 30-minute $Ni(CO)_4$ $LC_{50}$ reported for rats is about 85 mg $Ni/m^3$.

Repeated daily exposure for three months (seventy-one 6-hour periods) to concentrations averaging 9 mg $Co/m^3$ resulted in no chronic changes in rats, guinea pigs and dogs. Early deaths were due to acute effects. The lungs of animals sacrificed immediately after exposure contained nodules and aggregates of foam cells which were not found in animals sacrificed three and six months later. A single exposure six months later caused eosinophilic pneumonia in one of three guinea pigs.

Chemical analyses of the lungs and urine of exposed animals revealed that there is a rapid turnover of the bulk of the inhaled cobalt hydrocarbonyl. A substantial fraction was found in the urine within one day after exposure. The urinary cobalt level dropped by roughly one-half for each day after removal from exposure. A definite and persistent elevation of hemoglobin was observed in the rats exposed to cobalt hydrocarbonyl.

Kincaid and co-workers (106) investigated the toxicity of dicobalt octacarbonyl administered orally, percutaneously and subcutaneously to experimental animals. Their results indicate a low degree of toxicity for this compound by these routes.

## LITERATURE CITED


1. Mellin, G. W., and Katzenstein, M., *New Engl. J. Med.*, **267**, Part 1, p. 1184 and Part 2, p. 1238 (1962)
2. Somers, G. F., *Brit. J. Pharmacol.*, **15**, 111 (1960)
3. Editorial, *Can. Med. Assoc. J.*, **86**, 462 (1962)
4. Kunz, W., Heller, H., and Muckter, H., *Arzneimittel-Forsch.*, **6**, 426 (1956)
5. Burley, D. M., Dennison, T. C., and Harrison, W., *Practitioner*, **183**, 57 (1959)
6. Frenkel, H., *Med. Welt*, **18**, 970 (1961)
7. Burley, D. M., *Med. World*, **93**, 26 (1960)
8. Hayman, D. J., *Brit. Med. J.*, **2**, 1499 (1961)
9. Letters to editor, *Lancet*, **2**, 1262 (1961)
10. Cohen, S., *Am. J. Psychiat.*, **116**, 1030 (1960)
11. Lasagna, L., *J. Chronic Diseases*, **11**, 627 (1960)
12. Gray, R. W., Coats, E. A., Pogge, R. C., and MacQuiddy, E. L., *Diseases Nervous System*, **21**, 392 (1960)
13. Kuhn, W. L., and Van Maanen, E. F., *J. Pharmacol. Exptl. Therap.*, **134**, 60 (1961)
14. *A.M.A. News*, p. 10 (Aug. 20, 1962)
15. *Merck Index of Chemicals and Drugs*. 7th ed. (Rahway, Merck, N. J., 1960)
16. Wm. S. Merrell Co., letter to all Physicians, August 10, 1962
17. Taussig, H. B., *Sci. Am.*, **207**, 29 (1962)
18. Robertson, W. F., *Brit. Med. J.*, **1**, 792 (1962)
19. Alexander, I. R. W., *Brit. Med. J.*, **2**, 1434 (1961)
20. Hausmann, W., and Karlish, A. J., *Brit. Med. J.*, **2**, 1063 (1961)
21. Kendell, R. E., *Brit. Med. J.*, **2**, 1289 (1961)
22. Simpson, J. A., *Brit. Med. J.*, **1**, 55 (1962)
23. Lillicrap, D. A., *Brit. Med., J.*, **1**, 477 (1962)
24. Meade, B. W., and Rosalki, S. B., *Brit. Med. J.*, **2**, 1223 (1961)
25. Florence, A. L., *Brit. Med. J.*, **2**, 1954 (1960)
26. Raffauf, H. J., *Deut. Med. Wochschr.*, **86**, 935 (1961)
27. Cohen, S., *New Engl. J. Med.*, **266**, 1268 (1962)
28. Howe, P., *Brit. Med. J.*, **2**, 1084 (1961)
29. Fullerton, P. M., and Kremer, M., *Brit. Med. J.*, **2**, 855 (1961)
30. Powell-Tuck, G. A., *Brit. Med. J.*, **2**, 1151 (1961)
31. Scheid, W., Wieck, H. H., Stammler, A., Kladetzky, A., and Gibbels, E., *Deut. Med. Wochschr.*, **86**, 938 (1961)
32. Voss, R., *Muench. Med. Wochschr.*, **103**, 1431 (1961)
33. Cahill, P. K., *Brit. Med. J.*, **2**, 1223 (1961)
34. Simpson, J. A., *Brit. Med. J.*, **2**, 1287 (1961)
35. Lenz, W., and Knapp, K., *Arch. Environ. Health*, **5**, 100 (1962)
36. Wiedemann, H. R., *Med. Welt*, **18**, 1863 (1961)
37. *Medical Tribune*, **3**, 23 (Nov. 23, 1962)
38. Williams, R. T., *Lancet*, **I**, 723 (1963)
39. Evans, T. N., *Ind. Med. Surg.*, **32**, 87 (1963)
40. Millen, J. W., *Lancet*, II, 599 (1962)
41. *Lancet*, **I**, 705 (1963)
42. Hale, F., *Texas J. Med.*, **33**, 228 (1937)
43. Warkany, J., and Nelson, R. C., *Anat. Record*, **79**, 83 (1941)
44. Giroud, A., Lefebvres, J., Prost, H., and Dupuis, R., *J. Embryol. Exptl. Morphol.*, **3**, 1 (1955)
45. Nelson, M. M., Wright, H. V., Baird, C. D. C., and Evans, H. M., *Proc. Soc. Exptl. Biol. Med.*, **92**, 554 (1956)
46. Warkany, J., *Am. J. Diseases Children*, **66**, 511 (1943)
47. Cheng, D. W., Chang, L. F., and Bairnson, T. A., *Anat. Record*, **129**, 167 (1957)
48. Kalter, H., and Warkany, J., *J. Exptl. Zool.*, **136**, 531 (1957)
49. Nelson, M. M., Baird, C. D. C., Wright, H. V., and Evans, H. M., *J. Nutr.*, **58**, 125 (1956)
50. Nelson, M. M., Asling, C. W., and Evans, H. M., *J. Nutr.*, **48**, 61 (1952)
51. Sansone, G., and Zunin, C., *Acta Vitaminol.*, **8**, 78 (1954)
52. Nelson, M. M., Wright, H. V., Baird, C. D. C., and Evans, H. M., *J. Nutr.*, **62**, 395 (1957)
53. Pinsky, L., and Fraser, F. C., *Biol. Neonatorum.*, **1**, 106 (1959)
54. Murphy, M. L., *Ciba Found. Symp. Congenital Malformations, London* (1960)
55. Kalter, H., and Warkany, J., *Am. J. Pathol.*, **38**, 1 (1961)

56. Giroud, A., and Martinet, M., *Arch. Franc. Pediat.*, **12**, 292 (1954)
57. Giroud, A., and Martinet, M., *Arch. Franc. Pediat.*, **16**, 971 (1959)
58. Thiersch, J. B., *Proc. Soc. Exptl. Biol. Med.*, **94**, 33 (1957)
59. Thiersch, J. B., *Proc. Soc. Exptl. Biol. Med.*, **94**, 40 (1957)
60. Murphy, M. L., Del Moro, A., and Lacon, C., *Ann. N. Y. Acad. Sci.*, **68**, 762 (1958)
61. Haskin, D., *Anat. Record*, **102**, 493 (1948)
62. Murakami, U., Kameyama, Y., Majima, A., and Sakurai, T., *Ann. Rept. Res. Inst. Environ. Med.* (Nagoya Univ.) **9**, 71 (1961)
63. Warkany, J., and Schraffenberger, E., *Am. J. Roentgenol. Radium Therapy Nucl. Med.*, **57**, 455 (1947)
64. Smith, A. U., *J. Embryol. Exptl. Morphol.*, **5**, 311 (1957)
65. Fillipi, B., and Mela, V., *Minerva Chir.*, **11**, 1314 (1957)
66. Fillipi, B., and Mela, V., *Minerva Chir.*, **12**, 1047 (1957)
67. Nishimura, H., and Nakai, K., *Proc. Soc. Exptl. Biol. Med.*, **104**, 140 (1960)
68. Warkany, J., and Takacs, E., *Am. J. Pathol.*, **35**, 315 (1959)
69. Nishimura, H., and Nakai, K., *Science*, **127**, 877 (1958)
70. *New World of Chemicals*, *Chem. Eng. News*, **36**, 44 (1958)
71. *Metal Organic Organometallics*, *Ind. Eng. Chem.*, **49**, 44A (1957)
72. Bamford, C. H., Levi, D. L., and Newitt, D. M., *J. Am. Chem. Soc.*, **68**, 2204 (1946)
73. Pitzer, K. S., and Gutowsky, H. S., *J. Am. Chem. Soc.*, **68**, 2204 (1947)
74. Davidson, N., and Brown, H. C., *J. Am. Chem. Soc.*, **64**, 316 (1942)
75. Knap, J. E., Leech, R. E., Reid, A. J., and Tamplin, W. S., *Ind. Eng. Chem.*, **49**, 874 (1957)
76. Ziegler, K., Gellert, H. G., et al., *Brennstoff-Chem.*, **33**, 193 (1952)
77. *Triethyl Aluminum and Trimethyl Aluminum* (Technical Data Bulletin Ethyl Corp., New York, 1957)
78. Stoner, H. B., Barnes, J. M., and Duff, J. I., *Brit. J. Pharmacol.*, **10**, 16 (1955)
79. Barnes, J. M., and Stoner, H. B., *Brit. J. Ind. Med.*, **15**, 15 (1958)
80. Lyle, W. H., *Brit. J. Ind. Med.*, **15**, 193 (1958)
81. Elsea, J. R., and Paynter, O. E., *A.M.A. Arch. Ind. Health*, **18**, 214 (1958)
82. Bartalini, E., *Med. Lavoro*, **50**, 338 (1959)
83. Alajouanine, T., Derobert, L., and Thieffry, S., *Rev. Neurol.*, **98**, 85 (1958)
84. Lewis, C. E., *J. Occup. Med.*, **2**, 183 (1960)
85. Cremer, J. E., *Biochem. J.*, **67**, 87 (1957)
86. Cremer, J. E., *Biochem. J.*, **68**, 685 (1958)
87. Aldridge, W. N., *Biochem. J.*, **69**, 367 (1958)
88. Stoner, H. B., and Threlfall, C. J., *Biochem. J.*, **69**, 376 (1958)
89. Ansell, G. B., and Dohmen, H., *J. Neurochem.*, **1**, 150 (1956)
90. Dawson, R. M. C., and Richter, D., *Proc. Roy. Soc. London, Ser. B*, **137**, 252 (1950)
91. Kurland, L. T., Faro, S. N., and Siedler, H., *World Neurol.*, **1**, 370 (1960)
92. Hook, O., Lundgren, K. D., and Swensson, A., *Acta Med. Scand.*, **150**, 131 (1954)
93. Greco, A. R., *Riv. Neurolbiol.*, **3**, 515 (1930)
94. Swensson, A., Lundgren, K., and Lindstrom, O., *A.M.A. Arch. Ind. Health*, **20**, 567 (1959)
95. Friberg, L., *A.M.A. Arch. Ind. Health*, **20**, 42 (1959)
96. Uchida, M., Hirakawa, K., and Inoue, T., *Kumamoto Med. J.*, **14**, 171 (1961)
97. Uchida, M., Hirakawa, K., and Inoue, T., *Kumamoto Med. J.*, **14**, 181 (1961)
98. Hirakawa, K., Inoue, T., and Uchida, M., *Kumamoto Med. J.*, **14**, 192 (1961)
99. Davis, R. K., Horton, A. W., Larson, E. E., and Stemmer, K. L., *Arch. Environ. Health*, **6**, 473 (1963)
100. Hirschler, D. A., Gilbert, L. F., Lamb, F. W., and Niebylski, L. M., *Ind. Eng. Chem.*, **49**, 1131 (1957)
101. Cremer, J. E., *Brit. J. Ind. Med.*, **16**, 191 (1959)
102. Cremer, J. E., *Ann. Occup. Hyg.*, **3**, 226 (1961)
103. Cremer, J. E., and Callaway, S., *Brit. J. Ind. Med.*, **18**, 277 (1961)
104. Cremer, J. E., *Nature*, **195**, 607 (1962)
105. Palmes, E. D., Nelson, N., Laskin, S., and Kuschner, M., *Am. Ind. Hyg. Assoc. J.*, **20**, 453 (1959)
106. Kincaid, J. F., Strong, J. S., and Sunderman, F. W., *A.M.A. Arch. Ind. Hyg. Occup. Med.*, **10**, 210 (1954)

# CONTENTS