THE USE OF PROTOTYPE COMPOUNDS TO STUDY NEUROTOXICITY: A CASE STUDY OF THE ORGANOTINS

Deliverable Number 2595

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This deliverable is an executive summary of a number of published experiments. The publications are referenced in this summary and are contained in the Appendices in full length and annotated form.

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Executive Summary

The potential for a large number of chemicals to be neurotoxic poses a problem for a number of the EPA program offices charged with regulating chemicals. Few of the 65,000 chemicals used in daily commerce have been assessed for neurotoxic potential but of the chemicals that have been evaluated the Office of Technology Assessment (OTA) estimates at least 25% are neurotoxic (OTA, 1984). Given the potential for many chemicals to have neurotoxic actions there is a critical need for neurotoxicity risk assessment. The Neurotoxicology Division (NTD) is the focal point for neurotoxicology within EPA and is charged with the responsibility of devising means to detect and characterize neurotoxicity. The primary goal of the Division is to provide the scientific basis and technological means to be able to predict whether or not an environmental agent will produce neurotoxicity in humans. In pursuit of this goal the division is organized in a multidisciplinary fashion with research focused on six specific areas (1) methods development and validation, including evaluation of existing methods, design and evaluation of new methods, and development of testing strategies; (2) determination of the significance of variables that influence risk assessment based on animal data, including environmental and organismal variables; (3) developmental neurotoxicology, which evaluates the effects of developmental exposure on structure and function of the nervous system; (4) research leading to a reduction in uncertainties associated with quantitative dose-response determinations, including exposure scenarios, compensation or adaptation during repeated dosing; (5) research leading to a greater conceptual understanding of the neural substrate underlying neurobiological endpoints; and (6) studies on specific neurotoxic agents, including heavy metals, pesticides, industrial chemicals, and hazardous air pollutants. The use of known or prototype neurotoxic chemicals has figured prominently in several of these research areas. The research areas most impacted by the use of TET and TMT as prototype neurotoxicants has been those of methods development, developmental neurotoxicology and specific agent neurotoxicity characterization. Organotin research has had a minor impact on how environmental / organismal variables and exposure scenarios impact the assessment of neurotoxicity and have been least utilized in exploring mechanistic / conceptual issues in neurotoxicology.

This deliverable summarizes the body of work conducted by NTD utilizing the known or prototype neurotoxicants triethyltin (TET) and trimethyltin (TMT). Both chemicals are triorganotin compounds and their study provides for interesting structure-activity comparisons. TET has long been known as an agent that damages myelin but leaves neurons unharmed while TMT causes neuronal loss in certain brain areas. Both compounds damage the nervous system and are described as neuropathic. By definition a compound that causes structural damage to the nervous system is considered a neurotoxicant. A limited portion of the work was devoted to a characterization of the neurotoxicity associated with tributyltin (TBT), an organotin of environmental concern. NTD investigators have produced 56 papers on the organotin compounds of which 52 are research papers and the remaining 4 are reviews. Although both were known for their ability to cause specific types of structural damage to the nervous system the lack of a thorough characterization of their neurotoxic profiles in the adult and developing rodent prompted many of the NTD investigators to determine the specific characteristics of the neurotoxicity associated with acute exposure to TET and TMT. TET and TMT cause cellular damage to the CNS and thus it is not surprising that many of the NTD papers included a neuroanatomical assessment. Neuroanatomy was included as an endpoint of study in its own right or was included to verify the morphological damage caused by the organotin compound being studied. The use of neuroanatomical assessment as an adjunct was particularly prevalent in papers using either compound as part of a methods development strategy. These anatomical assessments ranged from the simple examination

of brain and brain area weights to more sophisticated neuroanatomical methods involving an evaluation of brain tissue at the light and electron microscopic level.

An examination of the NTD database on these two organotin compounds suggests the known CNS actions of TET and TMT have been most profitably exploited by the disciplines of behavior, electrophysiology and biochemistry in the areas of methods development. All these disciplines within NTD have used TET and TMT as representatives of the class of neurotoxicants that produce structural damage to the nervous system. Most investigators chose an acute or semiacute dosing regimen because morphological alterations can be demonstrated with both compounds after limited exposure. The working hypothesis for NTD investigators was that a procedure or test being developed to detect neurotoxicity should be able to identify a known neurotoxicant. Thus, these studies have provided critical elements in the body of data used to shape many of the guidelines proposed or being developed for neurotoxicity assessment. Both were used to cause defined damage to aid in the development of new methods as well as in comparisons of existing methods for their ability to detect neurotoxicity. Thus, data from these organotin studies have directly or indirectly had an impact on several guidelines. These have included the proposed sensory evoked potential, neuropathology, motor activity, functional observation battery test guidelines and to a lesser extent the developmental neurotoxicology testing guideline. In the future TET and TMT will serve as validation compounds that will aid in structuring the test guideline to identify neurotoxicants causing learning and memory deficits.

Developmental neurotoxicity research at NTD was also a primary beneficiary of the use of these prototype neurotoxicants. Studies with both compounds have shown the feasibility of using a model of early postnatal exposure to assess the risk of developmental neurotoxicity, although TET was utilized more often than TMT. These investigations have demonstrated the surprising and permanent impact a chemical can have on brain development after a limited exposure that does not alter somatic growth. Developmental neurotoxicants can be identified using this early postnatal model which conveniently avoids many of the complications inherent to *in utero* exposure models. NTD research has demonstrated the feasibility of using behavioral measures, such as motor activity and acoustic startle, to identify abnormal development of the CNS. Significant progress in the development of biochemical methods to identify neurotoxicants also resulted from these studies. Radioimmunoassays have been developed for proteins associated with the different cell types of the CNS. The ontogeny of certain developmental processes is mirrored in the developmental profiles of these proteins and perturbation of these processes by neurotoxic insult is detected by an alteration in these profiles. In particular, it was determined that assays of glial fibrillary acidic protein (GFAP), a protein associated with astrocytes which increases in response to injury, may be applicable to first tier testing. Developmental studies with both TET and TMT indicated that GFAP could be used to identify compounds able to damage developing CNS as readily as they identified those compounds damaging the adult brain. Developmental investigations with TET and TMT also demonstrated the utility of a simple morphological measurement, brain weight, in identifying agents that can disrupt CNS development. Finally, because NTD was actively investigating the issue of the neurotoxicity associated with TET and TMT it was possible to mount a rapid response to program office inquiries regarding the possible developmental neurotoxicity of TBT, an organotin of environmental concern.

Methods development has proceeded in concert with an expansion of the database on the CNS effects associated with TET and TMT and this existing database is a key element in future research. This large body of work can be exploited in future research directed at reducing uncertainty in risk assessment. Future efforts should be targeted to an exploration of issues related to exposure scenarios, quantitative dose-response relationships and organismic / environmental variables that will aid not only in reducing the uncertainty

in the prediction of neurotoxicity but will aid also in a greater conceptual understanding of neurotoxicity and its mechanisms of action. Further, the specific structural alterations caused by these organotins can be used to profitably explore basic function and organization of the CNS as well as the relationshp between systemically induced neurotoxicant damage and subsequent behavioral / functional alterations. In this way NTD will continue to make profitable use of the known neurotoxic profiles of TET and TMT. In summary, prototype neurotoxicants have and will continue to play a pivotal role in the NTD goal of providing the means for the prediction of neurotoxicity.

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INTRODUCTION

Rationale for the Use of Prototype Compounds - The public is exposed to a large number of chemicals - there are at least 65,000 used in daily commerce. However, few of these chemicals have been evaluated for their neurotoxic potential. Twenty-five percent of the chemicals that have been adequately evaluated have neurotoxic action (OTA, 1984; 1990). Periodic episodes involving large scale exposure of humans to compounds such as tri-ortho-cresyl phosphate , methylmercury, Kepone and MPTP (Canon et al., 1978; Kidd and Langworthy, 1933; Langston et al., 1983; Takeuchi, 1968) with resulting severe debilitation due to their neurotoxic effects, has highlighted the devastating consequences of such exposures. In the last decade neurotoxicology has commanded a growing importance and the increased need for hazard identification in this area of toxicology has been noted by many groups and individuals (see Tilson, 1990 for a discussion of the issues involved) (Reiter, 1980; Tilson, 1990; OTA, 1984; 1990). Thus, it is critical for Environmental Protection Agency (EPA) as a regulatory agency to have at its disposal the means to both detect and characterize potential neurotoxicants.

The Neurotoxicology Division (NTD) is the focal point within the EPA for studying the effects of physical and/or chemical agents on the nervous system. Consistent with this mission is the overall objective of providing the necessary scientific basis and technological means to predict whether or not an environmental agent will produce neurotoxicity in humans. Prediction of neurotoxicity is an important goal but equally important is minimizing the uncertainty in such predictions. The general approach is to model human neurotoxic disease in laboratory animals and then use data collected in these species to make predictions about possible neurotoxic risks in humans. Investigators in NTD adapt, develop or refine existing methods in pursuit of this goal. The NTD approaches its mission in a multidisciplinary fashion and investigation occurs at all levels of the neuraxis, including neurobehavioral, neurochemical, neurophysiological and neuroanatomical. Whole animal, cellular and molecular techniques are all applied in pursuit of the desired objective. The field of neurotoxicology is a relatively young discipline and as a first step in this endeavor many investigators in the NTD have chosen to use known or prototype neurotoxicants to determine the feasibility of their particular approach for detecting and characterizing neurotoxicity. Protocols designed to detect neurotoxic actions of unknown compounds should detect known neurotoxicants, that is, known neurotoxiciants are used to "validate" the test method (see Sette, 1987 for a discussion of this issue). Also consistent with this plan of action is that many of the peer reviewers of the NTD program have strongly urged all the principal investigators to test and validate their methodology by using known neurotoxicants. In brief, prototype neurotoxicants were used and continue to be used as positive controls or reference chemicals. Initially, many investigators in NTD chose to use as positive controls the two organotins, triethyl- and trimethyltin. By definition any compound that causes structural damage to the nervous system is considered a neurotoxicant. When given systemically both TET and TMT produce damage to the CNS and consequently have been described for many years as having "neurotoxic properties" or as being "neuropathic". Although both organotins are described as "neurotoxic" they have distinctly different neurotoxicological profiles (see discussion below). Both have been demonstrated to have neurotoxic action in humans.

Definition of a Prototype Compound and Why Triethyltin and Trimethyltin Are Used as Prototype Neurotoxic Compounds - A prototype is something that can serve as a model or can be considered as an example of a particular type. One definition of neurotoxicity is an alteration in the structural integrity of the nervous system and by extension a compound that causes structural alterations would be considered a neurotoxicant. Accordingly, both triethyltin and trimethyltin are considered to be prototype neurotoxicants of the type that cause structural damage. The nervous system

damage associated with each compound is clearly documented (see Overview section) and thus both compounds appeared to be ideal candidates to aid in establishing the viability of a multidisciplinary approach in accomplishing the mission of the Neurotoxicology Division.

Overview of Organotin Compounds in General with Emphasis on Triethyltin and Trimethyltin - Organotins as their name implies are compounds characterized by the presence of at least one covalent carbon-tin bond; most organotins have a tetravalent structure (Snoeij et al., 1987). These compounds have wide industrial and agricultural applications as plastic stabilizers and catalysts, as miticides, algicides, bacteriocides, fungicides and insecticides, and as wood and textile preservatives. The first organotin compound was synthesized in 1852 by Lowig but wide spread commercial use of these compounds has occurred primarily since the 1950s. There are currently three major uses for the organotins: 1) heat stabilizers in polyvinylchloride polymers, 2) industrial and agricultural biocides (Van der Kerk and Juijten (1954), and 3) industrial catalysts in a number of chemical reactions (Van der Kerk, 1978). In recent years, tributyltin oxide (TBT) has been of particular enviornmental concern (see Laughlin & Linden, 1985 for a discussion). TBT is an extremely effective antifouling agent and its incorporation into marine paint to control growth of aquatic organisms has resulted in damage to more sensitive aquatic species such as oysters. In 1986 EPA began a special review of compounds containing TBT and their use is now more strictly regulated under the Organotin Antifouling Paint Control Act of 1988. The commercial uses of TET and TMT, as compared to TBT, have been quite limited and these two organotins are more generally regarded as research tools. Many excellent reviews on the chemistry and uses of the organotins as well as their enviornmental impact are available and these aspects of the organotins will not be discussed further (Duncan, 1980; Hall & Pinkney, 1985; Hallas et al., 1982; Laughlin & Linden, 1985; Luijten, 1971; Maguire et al., 1986; Piver, 1973; Ross, 1965; Snoeij et al., 1987)

The number and type of organic substituents determine the mammalian toxicity of the organotins with triorganotins being more toxic than monoorganotins. However, toxicity within a given class of organotins also varies and is determined by the number of carbon atoms per side chain (Snoeij et al., 1987). Thus, in the series of triorganotins, the lower homologs, TET and TMT, are the most toxic while trioctyltin has little mammalian toxicity (compare oral LD50 values in Table 1). The short-chain alkyltins, TET and TMT, are the most neurotoxic while intermediate chain length compounds, such as tri-n-proplytin or TBT are immunotoxic (Snoeij et al., 1985). Until quite recently the acute toxic profile of both TET and TMT has been described as one of trembling, irritation, twitching, loss in body weight and progressive paralysis (Reiter & Ruppert, 1984; Tan and Ng, 1977). However, it is now apparent that these two organotins have strikingly different pathological profiles in adult organisms ((Brown et al., 1979).

TRIETHYLTIN - An extensive episode of poisoning by TET occurred in France (Barnes & Stoner, 1959) resulting from the accidental inclusion of TET and monoethyltin in a marketed product (Stalinon - preparation of diethyltin and linoleic acid) used to treat skin infection. One hundred of the 219 persons poisoned died with the pathological hallmark of this exposure being cerebral edema. Experimental exposure to TET results in interstitial edema that is found throughout the white matter of both brain and spinal cord and is the result of splitting and vacuolation of the myelin sheath at the interperiod line. Cell loss does not appear to be a concommitant of TET intoxication (Magee et al, 1957). The myelin alterations and subsequent edema appear to be reversible if a nonlethal dose is used and exposure is terminated.

Table 1. Acute oral LD_{50} values for various organotin compounds in the rat[a]

Compound	LD_{50}
mono-n-butyltin trichloride	2200; 2300
di-n-butytin 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]From Snoeij et al., 1987.

TRIMETHYLTIN - No cerebral edema is found after exposure to TMT but rather the pathological consequences of TMT intoxication consists of neuronal damage primarily within the hippocampus, amygdala, pyriform cortex, and neocortex (Brown et al., 1979; Bouldin et al., 1981). Human exposure (Fortemps et al., 1978; Ross et al., 1981) results in seizures, disorientation, insomnia, mental confusion, memory and learning impairments, anorexia and emotional lability. These symptoms are suggestive of neurotoxic action but no postmortem pathology data is available for humans. The vulnerability of the hippocampus is evident in a variety of species including primates (Brown et al., 1984). The pattern of hippocampal cell loss in the immature and adult rat is particularily striking (see photomicrographs in Brock & O'Callaghan, 1987; Miller & O'Callaghan, 1984).

RESULTS

Relationship of the Investigation of Organotin Compounds to the Overall Goals of NTD - Research in the Neurotoxicology Division focuses on the following specific areas: (1) methods development and validation, this can include the evaluation of existing methods, design and evaluation of new methods, and development of testing strategies; (2) determination of the significance of variables that influence risk assessment based on animal data, including environmental and organismal variables; (3) developmental neurotoxicology, which evaluates the effects of developmental exposure on structure and function of the nervous system; (4) research leading to a reduction in uncertainties associated with quantitative dose-response determinations, including exposure scenarios, compensation or adaptation during repeated dosing; (5) research leading to a greater conceptual understanding of neurotoxicology, including mechanism of action and a clear understanding of the neural substrate underlying neurobiological endpoints; and (6) studies on specific neurotoxic agents that can include heavy metals, pesticides, industrial chemicals, etc. The 56 studies conducted by NTD on the organotins (published or cleared) are listed in the Appendix in annotated form. This annotation includes information regarding the specifics of the study as follows: compound and dosing information; subject information including species, sex, age; whether or not a time course was performed; the method or methods employed; the research area or areas addressed; and a brief snynopsis of the research. Fifty-two of these papers are research studies and the remaining 4 are reviews; in this document all are referred to by the number they have in Appendix A.

As expected because of the known neurotoxic nature of TET and TMT, exposure to either resulted in anatomical, behavioral, biochemical and electrophysiological alterations. Exposure at any age produced deficits. Adults treated with TET showed reversible brain edema and motor function deficits as well as alterations in evoked potential endpoints and deficits in thermoregulatory capabilities (2, 27, 44). Detectable levels of elemental tin are found in brain and ATPase activity is inhibited after adult exposure to TET (12, 13, 14, 21, 53). None of the studies determining the effects of adult exposure to TET examined the nervous system at the light or electron microscopic level. Conversely, many of the studies characterizing the effects of adult exposure to TMT examined brain structures using these techniques. The most dominant feature noted was cell loss in the pyramidal cell line of the hippocampus although neuronal damage was found in other brain areas (1, 3, 4, 6, 7, 8, 9, 10, 11, 15, 18, 22, 26, 38, 40, 41, 50, 55). The CNS damage caused by TMT can be detected by changes in neuronal and glial proteins associated with the specific cell types of the nervous system (1, 3, 38, 40). Although elemental tin levels are elevated in brain the distribution characteristics do not explain the regional susceptibility to this neurotoxicant (12, 14). After exposure to TMT spontaneous seizures occur and susceptibility to other agents causing seizures is increased (25, 26). Rats exposed to TMT as adults are hyperactive, self-mutilate, and have deficient learning / memory capabilities and altered arousal as indicated by changes in startle reflex and visual or somatosensory evoked potentials (4, 5, 15, 24, 26, 28, 30, 32, 34, 41, 47, 50, 55, 56).

Exposure of the neonate to either TET or TMT resulted in permanent brain weight decreases that are accompanied by behavioral alterations including hyperactivity as well as learning / memory deficits and a deficient reaction to stimulation (31, 32, 33, 35, 37, 39, 43, 45, 46, 48, 49, 51, 52, 54). Early postnatal exposure to TMT resulted in a pattern of pyramidal cell loss exactly like that found in adult (33, 35, 52). Although TET caused a permanent brain weight decrease with the greatest effect found in hippocampus no overt cell loss was evident (37, 39, 54). Alterations in adult electrophysiological endpoints were found after neonatal exposure to TET; similar studies have not been performed after neonatal exposure to TMT (23). Assays of neuronal and glial localized proteins indicated both compounds cause damage to neurons and myelin when administered early in development (33, 35, 37, 38, 39). An increase in glial fibrillary acidic protein, an astrocyte localized protein, accompanies this damage and indicates the developing CNS can respond to injury in the same manner as the adult CNS (1, 3, 33, 35, 37).

An organization of the 52 research papers by compound, sex and whether it was an adult or developmental study is presented in Table 2. Most of these papers are relevant to 1 or more research areas considered important to NTD (see Table 3 for a compilation of the NTD studies on the organotins and the specific research area or areas they address). The proportion of the 52 research papers utilizing a particular organotin compound, dosing regimen and species is presented in Figure 1. Figure 2 presents the proportion of the 56 studies employing anatomy, behavior, biochemistry, electrophysiology, analysis or review as the primary method. Papers emphasizing behavior as a primary endpoint often employed other endpoints as well and the proportion of these behavioral papers using an additional method is also presented in Figure 2.

The organization of the organotin papers in the above manner provides some interesting information regarding the use of these prototype compounds by the NTD. TMT was studied more often with 53% of the studies utilizing this compound as compared to 31% for TET. However, when age of the subject at dosing is taken into consideration it is evident that most of the TMT studies were conducted in adults (see Table 3). TET was used more often than TMT in studies concerning developmental neurotoxicity. Rarely (12%) were the effects of TMT and TET directly compared. A small percentage of the total output (2 papers) concerned a characterization of the developmental neurotoxicity of TBT. It is clear that the rat was the experimental subject of choice with 99% of the research performed with this species. Male rats were always used if the study was concerned with the effect of either TET or TMT in adults. With one exception (see Table 3) all developmental studies determined the neurotoxicity of TET and TMT in both male and female subjects; the 2 studies of TBT also utilized both sexes. Acute and subacute dosing regimens were preferred; none of the 52 research papers employed a chronic treatment regimen.

It is clear that behavioral methods were used more often in assessing and characterizing the neurotoxicity of the three compounds (see the pie chart on the left side of Figure 2). Almost half of the studies used behavioral methods with anatomy being the next choice but accounting for a much lower percentage of the total (only 16.7%). However, many of the investigators who used behavior as a primary endpoint also utilized additional methods. When this is considered, anatomical endpoints (60%) were preferred with biochemical, electrophysiological, or physiological methods being chosen less often (see the pie chart on the right side of Figure 2). When research area is considered it is clear that the bulk of the studies were concerned with either methods development or a specific characterization of the neurotoxicity associated with the organotins (Table 3). In many instances methods development and neurotoxicity characterization were conducted in the same study. Developmental neurotoxicity was also a research area of major interest with

Table 2. Breakdown of NTD Organotin Studies by Compound, Age and Sex.

	TET		TMT		TBT	
Sex	Adult	Developing	Adult	Developing	Adult	Developing
male only	2, **12**, **13**, **14**, 20, 21, 27, 29, **30**, 44, **53**	54	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, **12**, **14**, 15, 19, 22, 24, 25, 26, 28, 34, 38, 40, 41, 47, 50, **53**, 55, 56			
female only					**16**	
both sexes		**13**, 23, 31, **32**, 37, 39, 43, **45**, 46, 48, 51, **53**	30	**32**, 33, **45**, 49, 52, **53**		**16**, 36

References in bold type appear in more than one category.
References # 17, 18, 35, 42 are reviews.

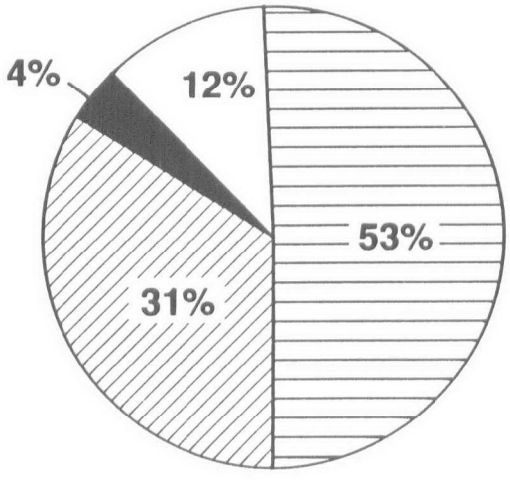
Table 3. NTD Research Areas and Organotin Publications by NTD Staff Addressing Each Area

Research Areas	Reference # in Appendix
Methods development and validation (**MD**)	1, 2, 3, **4, 5, 15**, 20, 22, 23, **24, 30, 31, 33 37, 38, 39, 40, 41,43,44,45, 47,49,52,55**
Environmental and organismal variables such as sex, age, etc. that influence risk assessment based on animal data (**EOV**)	11,13, **16**, 21, **26, 27, 30, 46, 53**
Developmental neurotoxicity - effects of exposure to toxicants during development on the structure and function of the nervous system (**DEV**)	**16, 31, 32, 33, 36, 37, 39,43, 45, 46, 48,49, 51, 52, 54**
Investigation of exposure scenarios, dosing regimens and other variables related to quanitative dose response determinations that will lead to a reduction in the uncertainty associated with extrapolation from laboratory animal data (**DR**)	12, **14, 32,40,44,48**
Conceptual understanding of neurotoxicity - includes research on mechanism of action and neural substrates underlying neurobiological endpoints (**CU**)	**4,5,14,19,33,37,50,51**
Investigation of a specific neurotoxic agent (**SN**)	6, 7, 9, 10, **15, 16, 24, 26, 27**, 28, 29, **30,32**, 34, **36, 38,43, 44, 47, 49, 50, 52, 54, 55**, 56,

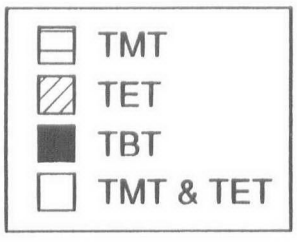
References in bold type are relevant to more than one research area. Abbreviations appearing under research area are those used in the annotated reference list contained in Appendix A.

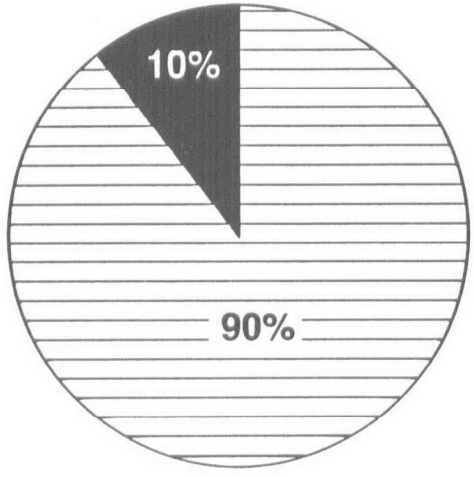
Figure 1.

Proportion of NTD Publications on Organotins By Compound, Dosing Regimen, and Species

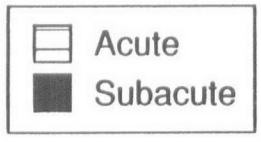


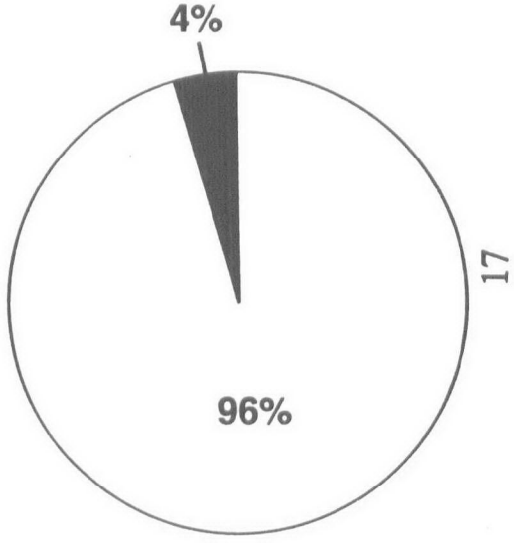
Compound





Dosing Regimen





Species

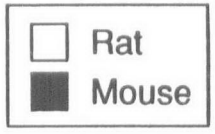
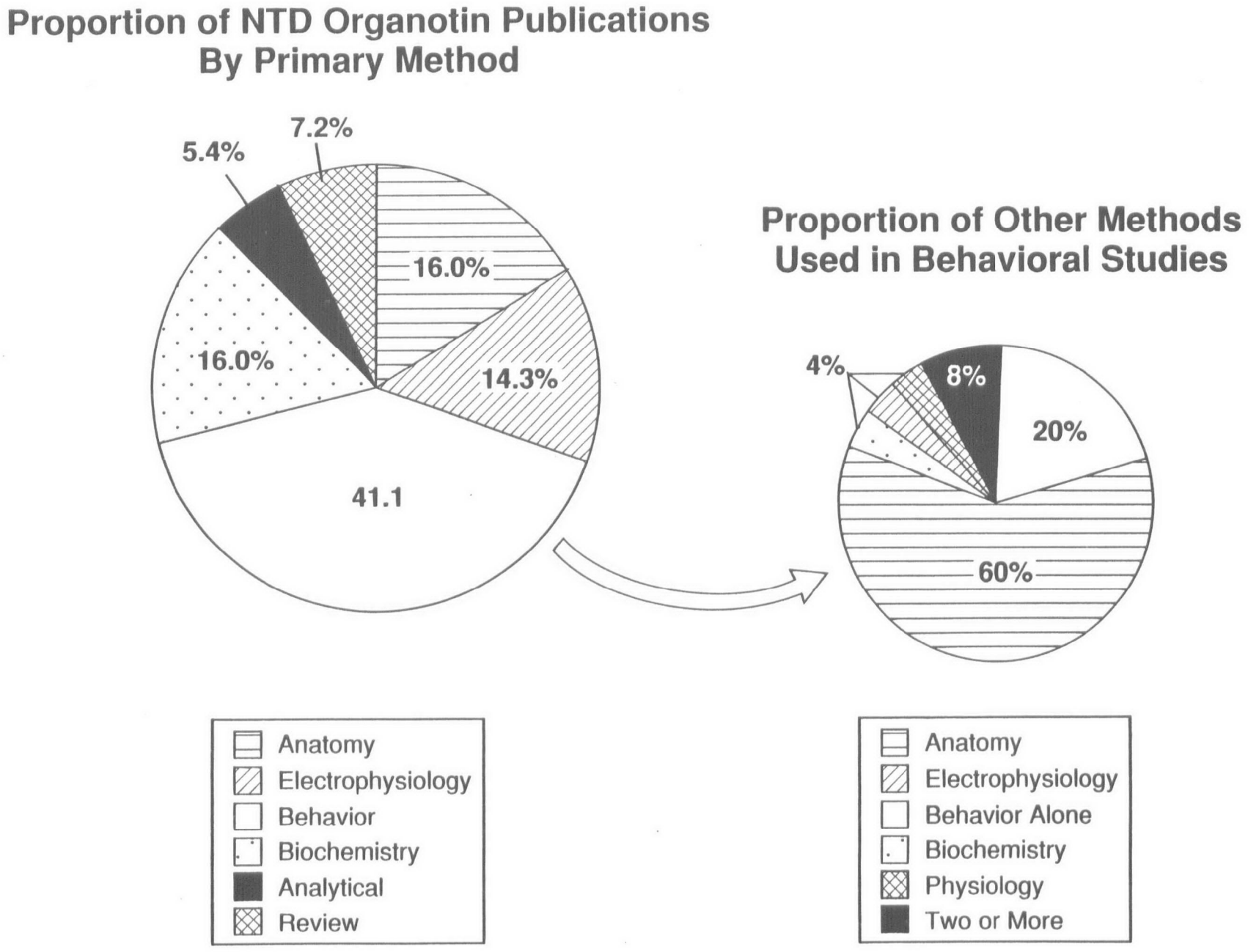


Figure 2.


Proportion of NTD Organotin Publications
By Primary Method
7.2%
5.4%
16.0%
14.3%
16.0%
41.1
Anatomy
Electrophysiology
Behavior
Biochemistry
Analytical
Review
Proportion of Other Methods
Used in Behavioral Studies
4%
8%
20%
60%
Anatomy
Electrophysiology
Behavior Alone
Biochemistry
Physiology
Two or More

methods development and neurotoxicant characterization counting heavily as topics of interest in these studies. However, few of the studies investigated how dosing regimens, exposure scenarios, or strain and species characteristics may influence the expression of the neurotoxicity associated with the organotins. Variables of this type were most often investigated in studies utilizing analytical techniques to determine the effects that age of the subject and speciation of the compound had on tin levels in tissue. Even fewer studies were directly concerned with investigating the mechanism of action of either TET or TMT.

CONCLUSION

This review of the NTD work on the neuropathic compounds, TET and TMT, indicates these prototypic neurotoxicants have aided and will continue to aid NTD in attaining its primary goal of providing the means for the assessment and prediction of neurotoxicity. Utilized as research tools since the formation of the NTD in 1980 these compounds have figured prominently in several important research areas of the NTD. Specifically, they have played pivotal roles in methods development and developmental neurotoxicology. In addition, they have been themselves the targets of detailed study that has more thoroughly characterized their neurotoxic attributes. The behavioral, biochemical and electrophysiological disciplines at NTD have benefited the most from the use of TET and TMT in methods development. Both have figured prominently as part of the database that has been utilized in the development of the formal guidelines for the evaluation of the effects of pesticides and other toxic substances on the nervous system. These guidelines include the use of a functional observational battery as well as tests for the assessment of motor activity (EPA, 1983; 1985; 1987). Further, both have figured prominently in establishing that visual evoked potentials (VEP) can be reliably altered by agents that cause structural damage to the CNS (Boyes & Dyer, 1988) and thus have been an important component of the database leading to the proposed test guidelines using sensory evoked potential methods (Boyes, 1990).

The use of these prototype compounds has also been particularily beneficial in the area of developmental neurotoxicology. Investigators at NTD using both TET and TMT have demonstrated the utility of the early postnatal exposure model for neurotoxicity assessment in the developing organism. Because compounds are administered soon after birth this model avoids many of the complicating factors associated with *in utero* exposure. However, this model still allows exposure during a period of significant CNS development. The postnatal model used in conjunction with both chemicals has proven valuable in demonstrating that functional aberrations accompany neurotoxic insult during development and thus have been instrumental in showing that behavioral procedures are one possible means of identifying developmental neurotoxicants. The postnatal model used in conjunction with both agents has shown the utility of assays of neurotypic and gliotypic proteins as a biochemical means of identifying deviations in critical developmental prcesses such as myelinogenesis and synaptogenesis. These assays also suggest TET damages neurons as well as myelin and thus further suggests that the neurotoxicity profiles of TET and TMT may be more similar during development than they are in the adult. However, there is a lack of research addressing the issue of neuronal damage following adult exposure to TET. Investigators at the NTD have also demonstrated that the simple morphological measure of brain weight can serve to identify a developmental neurotoxicant. The effects of TET and TMT on brain weight were particularily surprising. TET exposure in adults causes brain edema that results in an increase in brain weight. Further, this edema and weight increase are reversible when exposure is terminated. Developmental exposure to both TET and TMT cause permanent decreases in brain weight. The extensive investigation of the developmental neurotoxicity of TET and TMT allowed a rapid response of the NTD personnel to program office inquiries concerning the possible

neurotoxicity of TBT. This organotin, although of environmental relevance, had little significant developmental neurotoxicity compared to TET and TMT.

While the database on the developmental neurotoxicity of TET and TMT has been generated using postnatal exposures this information has been used in determining the tests to be used in the Collaborative Behavior Teratology Study (Adams et al., 1985) and in the structuring of the Developmental Neurotoxicity Guidelines (EPA, 1989). In both of these protocols compounds were or will be evaluated for neurotoxic potential during *in utero* exposure. The "generic" "developmental neurotoxicity protocol includes brain weight, motor activity and auditory startle. It has also been suggested that EPA personnel further evaluate the utility of the GFAP radioimmunoassay as a Tier 1 or "trigger" test for developmental neurotoxicants (Francis et al., 1990; Rees et al., 1990) . In summary, NTD personnel have used TET and TMT to show the utility of brain weight as a simple morphometric measurement that can provide an indication of brain dysmorphogenesis, the utility of GFAP as a general quantitative, biochemical indicator of damage to both the developing and adult nervous system, as well as the utility of the behavioral endpoints of motor activity and auditory startle as an indicators of abnormal development.

FUTURE RESEARCH DIRECTIONS

The major uses of the organotins, TET and TMT, have been as positive controls or known neurotoxicants in methods development and comparison of methods or as compounds for additional neurotoxicity characterization. Often methods development has proceeded in concert with an expansion of the database on the CNS effects associated with TET and TMT. This further neurotoxicity characterization as well as the already available database provides a large body of information that can be profitably exploited in future research directed at reducing uncertainty in risk assessment. However, information is lacking in certain areas and maximum use of the database will only occur if these data gaps are alleviated. The current database concerns only the acute or subacute effects of TET and TMT. This emphasis on acute rather than repeated exposure makes extrapolation from acute to chronic exposure regimens difficult. Future work should be directed towards exploring the problems that may be encountered in assessing neurotoxicity when exposure to a toxicant is chronic-low level rather than acute or subacute. Although developmental neurotoxicity has been a primary research area few studies have directly considered age or other organismic / environmental variables as factors that may affect the expression of the neurotoxicity associated with the organotins. There is a lack of comparable studies on the effects of TET and TMT in the adult. In particular there are few studies examining anatomical or behavioral effects after adult exposure to TET. Information on the age-related neurotoxicity of the organotins will aid in using the database to address structure - activity issues. A refinement of the current analytical techniques to allow a determination of the speciated form of the organotin rather than levels of elemental tin will aid in the investigation of distribution and metabolism of these compounds as well as structure - activity questions. Few studies have attempted to determine the mechanisms by which each of these organotins produces their selective profile of neurotoxicity. The lack of mechanistic studies is not surprising; a necessary precursor is a thorough characterization of the neurotoxicity of a compound as well as ways to manipulate its toxicity. The current data base provides the neurotoxicity characterization of TET and TMT. The next step in these mechanistic studies is to find ways to influence the expression of this neurotoxicity (e.g., pharmacological manipulations, etc.). Future research targeted to an exploration of issues related to exposure scenarios, quanitative dose-response relationships and organismic / environmental variables will aid not only in reducing the uncertainty in the prediction of neurotoxicity but will aid also in an exploration of the mechanisms of action of these compounds and a greater conceptual understanding of neurotoxicity. In

conclusion, prototype neurotoxicants have and will continue to play a pivotal role in the NTD goal of neurotoxicity prediction.

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Appendix A: Publications (Annotated) resulting from this Project.

1. Balaban, C. D., O'Callaghan, J. P., Billingsley, M. L. (1988). Trimethyltin induced neuronal damage in the rat: Comparative studies using silver degeneration stains, immunocytochemistry and immunoassay for neurotypic and gliotypic proteins. Neuroscience 26:337-361. (TMT OH- acute, 8 mg/kg IP; salt; rat, LE, male, 250-500 g; TC; **MD***; **ANAT**, BIOCHEM; extent and degree of CNS damage following TMT is area and time dependent, demonstrates utility of silver degeneration stain technique, radioimmunoassays of proteins associated with glial and neuronal constituents of nervous system in detecting CNS damage)

2. Boyes, W. K. and Dyer, R. S. (1983). Pattern reversal and flash evoked potentials following acute triethyltin exposure. Neurobehav. Toxicol. Teratol. 5: 571-577. (TET BR - acute, 0, 4.5, 6 mg/kg IP; salt; rat, LE, male, 220 - 360 g; No TC; **MD**; **ELEC**;pattern reversal rather than flash evoked potentials were better at detecting CNS alterations induced by TET)

3. Brock, T. O. and O'Callaghan, J. P. (1987). Quantitative changes in the synaptic vesicle proteins synapsin I and p38 and the astrocyte specific protein glial fibrillary acidic protein are associated with chemical-induced injury to the rat central nervous system. J. Neurosci. 7: 931-942. (TMT OH - acute, 0, 3, 6, 8, 9 mg/kg IV;base; rat, LE, male, 200-250 g; TC; **MD**; **BIOCHEM**, ANAT; because RIA values of gliotypic and neuronotypic proteins are altered in a dose- and time-dependent manner following damage induced by TMT they can be used to detect and characterize CNS damage induced by other toxicants)

4. Bushnell, P. J. (1990). Delay-dependent impairment of reversal learning in rats treated with trimethyltin. Behavioral and Neural Biology IN PRESS. (TMT OH - acute, 0, 7 mg/kg IV; base; rat, LE, male, 350 g; TC; **MD**, CU; **BEHAV**, ANAT; TMT-induced impairment in automaintained reversal learning was evident only if a delay was incorporated into task, behavioral deficit not completely related to morphological change)

5. Bushnell, P. J. (1988). Effect of delay, intertrial interval, delay behavior and trimethyltin on spatial delayed response in rats. Neurotox. Teratol. 10: 237-244. (TMT OH - acute, 0,7 mg/kg IV; base; rat, LE, male, 350 g; TC; **MD**, CU; **BEHAV**, BIOCHEM; spatial delayed response task validated as a measure of working memory and as a method for detection of toxicant-induced CNS damage, RIA of GFAP, a gliotypic protein that increases in response to CNS damage, used to quantify TMT-induced CNS injury)

6. Chang, L. W. and Dyer, R. S. (1983). A time-course study of trimethyltin induced neuropathology in rats. Neurobehav. Toxicol. Teratol. 5: 443-459. (TMT CL - acute, 0, 6 mg/kg OR; base, rat, LE, male, 60 - 80 d; TC; **SN**; **ANAT**; descriptive neuropathological assessment after TMT suggests limited damage to dentrate gyrus area of hippocampus; the time course for pyramidal cell loss in hippocampus is protracted, accompanied by gliosis and is more severe in the septal than temporal portions)

7. Chang, L. W. and Dyer, R. S. (1983). Trimethyltin induced pathology in sensory neurons. Neurobehav. Toxicol. Teratol. 5: 673-696. (TMT Cl - acute, 0, 6 mg/kg OR; base, rat, LE, male, 60 - 80 d; TC; **SN**; **ANAT**; TMT is generally considered to be a limbic system toxicant but descriptive neuropathological assessment at the light and electron microscopic level suggest damage to sensory neurons of retina, inner ear, dorsal root ganglia, olfactory and pyriform cortices)

8. 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. Alan R. Liss Series in Neurology and Neurobiology, pp. 275-290, Alan R. Liss, Inc, New York.(TMT - acute, 0, 6 mg/kg IP; base; rat, SD, male, 200 g; TC; **SN**; **ANAT**; descriptive neuropathological assessment at the light microscopic level with cresyl violet stain and modified Timm's method for heavy metal staining suggest neuronal damage induced in hippocampus by TMT is accompanied by a reduction or depletion of zinc in the mossy fibers of this area)

9. Chang, L. W. and Dyer, R. S. (1985). Septo-temporal gradients of trimethyltin-induced hippocampal damage. Neurobehav. Toxicol. Teratol. 7:43-49.(TMT CL - acute, 0, 6 mg/kg OR; base; rat, LE, male, 60 -80 d; TC; **SN**; **ANAT**; descriptive neuropathological assessment suggest damage by TMT to particular cells in hippocampus depends on the location of the cell within the hippocampal formation as well as subfield identity, granule cells are most affected at temporal pole, more pyramidal cell loss occurs in the septal region)

10. 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. (TMT Cl - acute, 0, 6 mg/kg OR; base; rat, LE, male, 60-80 d; No TC; **SN**; **ANAT**; descriptive neuropathological assessment suggest alterations in dentate gyrus basket cells are observable by 24 hrs after TMT using electron but not light microscopic techniques.)

11. Chang, L. W., Wenger, G. R., McMillan, D. E. and Dyer, R. S. (1983). Species and strain comparison of acute neurotoxic effects of trimethyltin in mice and rats. Neurobehav. Toxicol. Teratol. 5: 337-350. (TMT Cl - acute, 7.5 (rat) 3.0 (mouse) mg/kg.; base; rat, LE and SD; mouse, C57Bl/6 and BALB/c, male, 60-90 day; No TC; **EOV**; **ANAT**; descriptive neuropathological assessment suggests species and strain differences in areas of hippocampal damage and neurological signs, mice were more sensitive than rats and LE rats more sensitive than SD rats)

12. Cook, L. L., Heath, S. M. and O'Callaghan, J. P. (1984). Distribution of tin in brain subcellular fractions following the administration of trimethyl tin and triethyl tin to the rat. Toxicol. Appl. Pharmacol. 73: 564-568. (TET Br, TMT OH - acute, 6.0 mg/kg; base; rat, LE, male, 200 - 250 g; TC; **DR**; **ANALY**; distributional profile of tin in subcellular fractions of brain as measured by flameless atomic absorption spectrometry varied as a function of the organic moiety, tin levels were lower, persisted longer, and concentrated in mitochondrial fraction after TMT compared to TET)

13. Cook, L. L., Jacobs, K. S. and Reiter, L. W. (1984). Tin distribution in adult and neonatal rat brain following exposure to triethyltin. Toxicol. Appl. Pharmacol. 72: 75-81.(TET Br - acute, 0, 3, 6, 9 mg/kg IP; salt; rat, CD, male, 60 d or male & female, PND-5; TC; **EOV**; **ANALY**; tin levels as assessed by flameless atomic absorption spectrometry were evenly distributed across brain areas, rate of elimination did not vary as a function of age but concentration of tin in neonate brain declined faster than adult because of brain growth)

14. Cook, L. L., Stine, K. E., and Reiter, L. W. (1984). Tin distribution in adult rat tissues after exposure to trimethyltin and triethyltin. Toxicol. Appl. Pharmacol. 76: 344-348. (TET Br , TMT OH - acute 3, 6, 9 mg/kg IP (TET only 6 mg/kg), base; rat, LE, male, 60 d; TC; **DR**, CU; **ANALY**; tin levels and rates of elimination as assessed by flameless atomic absorption spectrometry were greater in all tissues following TET as compared to TMT, brain area distributions of tin following TMT were equivalent so distribution differences do not acount for the region-specific pathology associated with TMT)

15. Crofton, K., Dean, K., Menache, M. and Janssen, R. (1990). Trimethyltin effects on auditory function and cochlear morphology. Toxicol. App. Pharmacol. IN PRESS. (TMT OH - acute 3, 5, 7 mg/kg, base; rat, LE, male, 60 d; No TC; **MD**, SN; **BEHAV**, ELEC; ANAT; TMT causes histopathological changes in inner ear - cochlear hair cell loss - associated with auditory dysfunction detectable by auditory startle reflex and brainstem auditory evoked response methodology)

16. Crofton, K. M., Dean, K. F., Boncek, V. M., Rosen, M. B., Sheets, L. P., Chernoff, N. and Reiter, L. W. Prenatal or postnatal exposure to bis (tri-n-butyltin) oxide in the rat; Postnatal evaluation and behavior. Toxicol. App. Pharmacol. 97:113-123. (TBTO - subchronic 0, 2.5, 5, 10, 12, 16 mg/kg from GD 6 - 20 or acute 0,40,50,60 mg/kg PND5, salt; rat, LE, female & male; TC; **DEV**, SN, EOV; **BEHAV**, TERATOL, TOXICOL, ANAT;prenatal TBTO caused effects in Chernoff-Kavlock postnatal teratology screen only at maternally toxic doses, postnatal exposure caused brain weight decreases and behavioral alterations at only at doses causing pup mortality)

17. Dyer, R. S. (1987). Macrophysiological assessment of organometal neurotoxicity. In H. A. Tilson and S. B. Sparber (eds.) Neurotoxicants and Neurobiological Function: Effects of Organoheavy Metals. John Wiley and Sons, Inc. pp. 137-184. (TET, TMT - **REV**; ELEC; macrophysiological techniques such as EEG, evoked potential, kindling and afterdischarge, drug-induced, and maximal-electroshock seizures can be used to further characterize the neurotoxicity of TET and TMT)

18. Dyer, R. S. (1982). Physiological methods for assessment of trimethyltin exposure. Neurobehavior. Toxicol. Teratol. 4: 659-664. (TMT - **REV**; ELEC; gross physiological measures of limbic system function are not as efficient at detecting TMT damage as intrahippocampal evoked potentials, preliminary neuropathological descriptions are not adequate to direct physiological studies and result in an inadequate characterization of neurotoxicity)

19. Dyer, R. S. and Boyes, W. K. (1984). Trimethyltin reduces recurrent inhibition in rats. Neurobehav. Toxicol. Teratol. 6: 369-371. (TMT OH - acute 0, 5, 6 mg/kg OR,base; rat, LE, male;TC; **CU**; **ELEC**; TMT reduces recurrent inhibition in hippocampus within 2 hours of dosing suggesting that increased activity in mossy fibers is a result of reduced basket cell inhibition of the dentate granule cells)

20. Dyer, R. S. and Howell, W. E. (1982). Acute triethyltin exposure: effects on the visual evoked potential and hippocampal afterdischarge. Neurobehav. Toxicol. Teratol. 4: 259-266.(TET Br - subchronic 0, .188, .375, .75, 1.5 mg/kg/day IP for 6 days; rat, LE, male; TC; **MD**; **ELEC**; both visual evoked response (VER) and hippocampal afterdischarge (AD) can detect acute effects of TET, VER changes indicate myelinopathy and/or CNS depression, threshold change was the most sensitive AD measure)

21. Dyer, R. S. and Howell, W. E. (1982). Triethyltin: ambient temperature alters visual system toxicity. Neurobehavior. Toxicol. Teratol. 4: 267-271.(TET Br - acute 0,3,6,9 mg/kg IP; rat, LE, male; TC; **EOV**; **ELEC**; TET caused dose-related hypothermia and increased visual evoked response latencies, preventing hypothermia by maintainance in a warmer temperature worsened electrophysiological signs of neurotoxicity)

22. Dyer, R. S., Deshields, T. L. and Wonderlin, W. F. (1982) Trimethyltin-induced changes in gross morphology of the hippocampus. Neurobehav. Toxicol. Teratol. 4:141-147. (TMT Cl - acute, 0,5,6,7 mg/kg OR; base; rat, LE, male, 50 - 60 d; TC; **MD**; **ANAT**; comparison of simple anatomical methods (length of pyramidal cell line and thickness of dentate gyrus (DG), granule cell layer, CA1, CA3, intrahilar areas of DG) to evaluate the morphological changes caused by TMT indicate the most clearly dose-related was length of pyramidal cell line from CA1-CA3)

23. Dyer, R. S., Howell, W. E. and Reiter, L. W. (1981). Neonatal triethyltin exposure alters adult electrophysiology in rats. Neurotoxicology 2: 609-623. (TET Br - acute, 0, 3, 6, 9 mg/kg IP; salt; rat, SD, male & female, PND5; No TC; **MD**; DEV; **ELEC**; visual evoked potentials (VEPS); when tested as adults hippocampal afterdischarges, picrotoxin and pentylenetetrazol seizure susceptibility indices show VEP is sensitive to perinatal toxicant exposure)

24. Dyer, R. S., Howell, W. E. and Wonderlin, W. F. (1982). Visual system dysfunction following acute trimethyltin exposure in rats. 4: 191-195. (TMT Cl - acute, 0,4,5,6,7 mg/kg OR; base; rat, LE, male, 60 d; TC; **SN**, MD; **ELEC**; TMT causes visual system damage that is detected by visual evoked responses (VER), VER changes suggest alterations in retinal processing and arousal)

25. Dyer, R. S., Wonderlin, W. F. and Walsh, T. J. (1982). Increased seizure susceptibility following trimethyltin administration in rats. Neurobehav. Toxicol. Teratol. 4: 203-208. (TMT Cl - acute, 0, 5, 6, 7 mg/kg OR; base; rat, LE, male, 277 - 284 g; TC; **SN**, MD; **ELEC**; TMT increased seizure indices in all models (behavioral scoring, hippocampal and amygdaloid kindling, sensitivity to pentylenetetrazol-induced seizures, afterdischarge threshold) in a dose-related manner)

26. Dyer, R. S., Walsh, T. J., Wonderlin, W. F. and Bercegeay, M. (1982). The trimethyltin syndrome in rats. Neurobehav. Toxicol. Teratol. 4: 127-133.(TMT Cl - acute, 0, 1 - 10 mg/kg OR or IP; base; rat, LE, male, 200 - 600 g; TC; **EOV**, SN; **BEHAV**, TOXICOL, PHYSIOL, ANAT; TMT exposure causes unique syndrome that includes spontaneous seizures, tail mutilation, vocalization, weight and/or age appear to determine degree of toxicity (death and weight loss)

27. Gordon, C. J., Long, M. D. and Dyer, R. S. (1984). Effect of triethyltin on autonomic and behavioral thermoregulation of mice. Toxicol. Appl. Pharmacol. 73: 543-550. (TET Br - acute, 0, 2, 4, 6, 8 mg/kg IP; salt; mouse; BALB/c, male, young adult; TC; **EOV**, SN; **BEHAV**; PHYSIOL; TET results in hypothermia, no change in evaporative water loss, a decrease in metabolic rate and a preference for cooler temperatures suggesting altered thermoregulation)

28. Howell, W. E., Walsh, T. J. and Dyer, R. S. (1982). Somatosensory dysfunction following acute trimethyltin exposure. Neurobehav. Toxicol. Teratol. 4: 197-201. (TMT Cl - acute, 0, 7 mg/kg OR; base; rat, LE, male, 320 - 540 g; TC; **SN**; **BEHAV**, ELEC; TMT alters somatosensory function as shown by increased latency to respond to a heat stimulus, increased somatosensory evoked responses, but no change in peripheral nerve function [conduction velocity and threshold] and suggests TMT causes central but not peripheral damage)

29. Jacobs, K. S., Lemasters, J. J. and Reiter, L. W. (1983). Inhibition of ATPase activities of brain and liver homogenates by triethyltin (TET). In Developments in the Science and Practice of Toxicology, A. W. Hayes, R. C. Schnell and T. S. Miya, Eds. Elsevier Science Publishers, B.V. pp. 517-520. (TET Br - acute, 0, 6 mg/kg IP; rat, SD, male; TC; **SN**; **BIOCHEM**, ANALY; TET more effectively inhibits liver than brain ATPase when given in vivo)

30. MacPhail, R. C. (1982). Studies on the flavor aversions induced by trialkyltin compounds. Neurobehav. Toxicol. Teratol. 4: 225-230. (TMT OH - Repeated, 0, .625, 1.25, 2.5, 5.0 mg/kg x 3 IP; base; TET Br - acute, 0, .375, .75, 1.5, 3.0 IP or repeated, 0,.188, .375, .75, 1.5 mg/kg IP x 5 or 6 or acute, 0, 5 mg/kg IP; base; rat; LE, male (TET, TMT) female (TMT); TC; **MD**, EOV, SN; **BEHAV**; TET and TMT both result in flavor aversions (FA) that are dependent on both the dose and number of pairings of the toxicant and flavor, doses of TET or TMT required to produce FA are at least 25 - 45% of the LD-50 values, sex was not a factor in development of FA to TMT and familiarity with test chamber did not alter FA to TET)

31. Miller, D. B. (1984). Pre- and postweaning indices of neurotoxicity in rats: Effects of triethyltin (TET). Toxicol. Appl. Pharmacol. 72: 557-565. (TET Br - acute, 0, 3, 6 mg/kg IP; salt; rat, LE, male & female, PND5; TC; ;**DEV**, MD; **BEHAV**; ANAT; TET alters function during life span as evidenced by alterations in preweaning, juvenile and adult activity (automated and open-field) and learning (alleyway conditioning and radial arm maze), preweaning deficits can be detected by tailoring tasks to limited capabilities of neonates, TET causes permanent decreases in whole brain, hippocampus and cerebellum weights with hippocampus most affected)

32. Miller, D. B., Eckerman, D. A., Krigman, M. R. and Grant, L. D. (1982). Chronic neonatal organotin exposure alters radial-arm maze performance in adult rats. Neurobehav. Toxicol. Teratol. 4: 185-190. (TET sulfate or TMT OH semichronic 0, 0.3, 1.0 mg/kg OR PND 3-29, TMT 1.0 mg/kg every other day; rat; LE; male & female; No TC; **DEV**, SN, DR; **BEHAV**; rats given TET were more active, took longer to reach criterion and showed a transient deficit in accuracy when tested as adults in radial arm maze)

33. Miller, D. B. and O'Callaghan, J. P. (1984). Biochemical, functional and morphological indicators of neurotoxicity: Effects of acute administration of trimethyltin to the developing rat. J. Pharmacol. Exp. Ther. 231: 744-751.(TMT OH - acute, 0, 5, 6 mg/kg IP; base; rat, LE, male & female; PND5; TC; **DEV**, MD, CU; **BIOCHEM**, BEHAV, ANAT; TMT interferes with brain development as indicated by dose and time dependent alterations in a synapse-localized phosphoprotein, permanent decreases in brain, hippocampus and cerebellum weights, loss of hippocampal CA3-4 pyramidal cells and behavioral dyfunctions including hyperactivity and compromised learning (alleyway conditioning, passive avoidance, radial-arm maze;demonstrates utility of multi-endpoint and timepoint strategy for detection and characterization of neurotoxicity)

34. Myers, R. D., Swartzwelder, H. S. and Dyer, R. S. (1982). Acute treatment with trimethyltin alters alcohol self-selection. Psychopharmacology, 78: 19-22. (TMT CL - acute, 0, 7 mgl/kg OR; base; rat, LE, male; 348 -460 g; No TC; **SN**; **BEHAV**; ANAT; compared to controls TMT-treated rats consummed markedly less alcohol, regardless of concentration, whether selection occurred in the home-cage or a food contingent schedule-induced polydypsia situation, effects not due to changes in gustatory sensitivity but may be related to damage of forebrain structures)

35. O'Callaghan, J. P. and Miller, D. B. (1989). Assessment of chemically-induced alterations in brain development using assays of neuron- and glia-localized proteins. Neurotoxicol. 10:393-406. (TBT, TET, TMT - **REV**; **BIOCHEM**, ANAT; reviews the use of neuron- and glial-localized proteins in detecting neurotoxic insult to the developing nervous system; known neurotoxicants such as TET and TMT were used to damage developing brain; significant changes in these proteins can be observed in the absence of cytopathology or decreases in brain weight; the astrocyte-localized protein, glial fibrillary acidic protein, increases in response to chemically induced brain injury in the neonate as well as in the adult)

36. O'Callaghan, J. P. and Miller, D. B. (1988). Acute exposure of the neonatal rat to tributylin results in decreases in biochemical indicators of synaptogenesis and myelinogenesis. J. Pharmacol. Exp. Ther. 246: 394-402. (TBT oxide - acute, 0, 2, 3, 4, 5 mg/kg IP; base; rat, LE, male & female; PND5; TC; **DEV**, SN; **BIOCHEM**, ANAT; TBT caused decreases in the synapse-localized protein, p38, and the oligodendroglial protein, myelin basic protein, in hippocampus and cerebellum that did not persist into adulthood at doses that did not alter brain, thymus or body weight; shows neurotoxicity can be detected by assays of neurotypic and gliotypic proteins in the absence of overt changes in morphology, TBT is not as neurotoxic as TET or TMT)

37. O'Callaghan, J. P. and Miller, D. B. (1988). Acute exposure of the neonatal rat to triethyltin results in persistent changes in neurotypic and gliotypic proteins. J. Pharmacol. Exp. Ther. 244: 368-378. (TET Br - acute, 0, 3, 6 mg/kg IP; salt; rat, LE, male & female; PND5; TC; **DEV**, MD, CU; **BIOCHEM**, ANAT; TET caused permanent brain and brain area weight decreases, permanent alterations in proteins associated with specific developmental processes [e.g., synaptogenesis, myelinogenesis, etc] show utility of assays of neuro and gliotypic proteins in neurotoxicity assessment, increases in the astrocyte-associated protein, glial fibrillary protein, show developing nervous system responds to CNS damage in a manner similar to adult brain)

38. O'Callaghan, J. P. and Miller, D. B. (1984). Neuron-specific phosphoproteins as biochemical indicators of neurotoxicity: Effects of acute administration of trimethyltin to the adult rat. J. Pharmacol. Exp. Therap. 231: 736-743.(TMT OH - acute, 0, 6, 8, 9 mg/kg IV; base; rat, LE, male; 175-225 g; TC; **MD**, SN; **BIOCHEM**, ANAT; TMT caused dose- and time-related decreases in hippocampal phosphoproteins and changes were still evident 14 weeks following dosing and suggests neuronal phosphoproteins may be used as biochemical indicators of neurotoxicity)

39. 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. (TET Br - acute, 0, 3, 6 mg/kg IP; salt; rat, LE, male & female; PND5; TC; **DEV**, MD; **BIOCHEM**, ANAT; TET caused permanent dose-related decreases in brain weights and alterations in the phosphoprotein, myelin basic protein suggesting alterations in myelinogenesis)

40. O'Callaghan, J. P., Niedzwiecki, D. M. and Means, J. C. (1989). Variations in the neurotoxic potency of trimethyltin. Brain Research Bulletin 22: 637-642. (TMT OH or Cl - acute, 0, 8 mg/kg IV; base; rat, LE, male; 40 d; No TC; **DR**, MD; **BIOCHEM**, ANALY; 7 commercially available sources of TMT specified at greater than 95% pure produced varying degrees of CNS damage as revealed by changes in histology, hippocampal weight and concentration of the astrocyte protein, glial fibrillary acidic protein; analysis of samples for organotin content as well as contaminants confirmed that the decreases in neurotoxicity could be attributed to sample to sample variation in TMT content)

41. Peele, D. B., Farmer, J. D. and Coleman, J. E. (1989) Time-dependent deficits in delay conditioning produced by trimethyltin. Psychopharmacology 97: 521-528. (TMT OH - acute, 0, 8 mg/kg IV; base; rat, LE, male, 40 d; TC; **MD**; **BEHAV**, ANAT; TMT resulted in passive avoidance and taste aversion conditioning deficits when delay was incorporated as part of the test, TMT did not affect taste discrimination and because similar delay dependent effects were evident in both learning tests suggests these are true deficits in memorial processes rather than due to effects of TMT on sensory, motor or associative processes)

42. Reiter, L. W. and Ruppert, P. H. (1984). Behavioral toxicity of trialkyltin compounds: A review. Neurotoxicology 5: 177-186. (TMT, TET - **REV**; BEH; review of the symptomatology reported after human exposure to TET and TMT with a comparison of the functional or behavioral alterations that accompany exposure to TET and TMT in the adult nonhuman organism)

43. Reiter, L. W., Heavner, G. G., Dean, K. F. and Ruppert, P. H. (1981). Developmental and behavioral effects of early postnatal expousre to triethyltin in rats. Neurobehav. Toxicol. Teratol. 3: 285-293. (TET Br - acute, 0, 3, 6, 12 mg/kg IP; salt; rat, SD, male & female, PND5; TC; **DEV**, MD, SN; **BEHAV**, ANAT; tests for physical maturation, development of reflexes, motor coordination, motor activity accompanied by measurement of brain weight indicate TET interferes with CNS development as suggested by delayed development of rope descent and locomotion, persistent increases in motor activity as adults and a permanent brain weight decrease)

44. Reiter, L. W., Kidd, K., Heavner, G. and Ruppert, P. H. (1980). Behavioral toxicity of acute and subacute exposure to triethyltin in the rat. Neurotoxicology 2: 97-112. (TET Br - acute, 0, 1.5, 3.0 mg/kg SC or subchronic 0, 5, 10, 15, 20 mg/liter for 3 wks DW, 0, 8.4, 13.9, 17.3 mg/kg total intake; salt; rat, SD, male, 90-120 d; TC; **DR**, SN, MD; **BEHAV**, ANAT, TOXICOL; acute and subacute dosing with TET causes decrements in motor activity, open field behavior, acoustic startle response and landing foot spread that are reversible within 1 month after dosing ceases, BEHAV changes occurred as doses not affecting growth or reducing food and water intake; dose related increases in brain but not other organ weights accompany subacute exposure)

45. Ruppert, P. H., Dean, K. F. and Reiter, L. W. (1985). Development of locomotor activity of rat pups exposed to heavy metals. Toxicol. Appl. Pharmacol. 78: 69-77. (TET BR - acute, 0,4,5,6 mg/kg IP; salt; TMT OH - acute, 0, 4, 5, 6 mg/kg IP; base; rat; LE; male & female, PND5; TC; **MD**, DEV; **BEHAV**; treatment with TET and TMT caused hyperactivity to develop by the end of the preweaning period and suggest preweaning assessment of locomotor activity would be predictive of juvenile or adult activity changes)

46. Ruppert, P. H., Dean, K. F. and Reiter, L. W. (1984). Neurobehavioral toxicity of triethyltin in rats as a function of age at postnatal exposure. Neurotoxicol. 5: 9-22. (TET BR - acute, 0, 1.5, 3.0, 6.0 mg/kg IP; salt; rat; SD; male & female, PND1, 5, 10, 15; TC; **EOV**, DEV; SN; **BEHAV**,TOXICOL; ANAT; developmental toxicity depended on day of exposure, all exposure days resulted in preweaning growth retardation but only PND5 exposure produced a permanent brain weight decrease, increased seminal vesicle weights and behavioral deficits that included preweaning deficits in rope descent and adult hyperactivity)

47. Ruppert, P. H., Dean, K. F. and Reiter, L. W. (1984). Trimethyltin disrupts acoustic startle responding in adult rats. Toxicol. Lett. 22: 33-38. (TMT OH - acute, 0, 4, 5, 6 mg/kg IP; base; rat; LE; male, 60 d; TC; **MD**, SN; **BEHAV**; all doses of TMT reduced the number of responses, response amplitude and sensitization by background noise while response latency was increased, deficits occurred within 2 hrs of dosing and were still evident 4 weeks later, because hippocampal lesions do not produce this pattern of deficits it is unlikely TMT-induced limbic system damage can account for these effects, more likely they are due to damage to the primary auditory startle circuit)

48. Ruppert, P. H., Dean, K. F. and Reiter, L. W. (1983). Comparative developmental toxicity of triethyltin using a split-litter and whole-litter dosing. J. Toxicol. Environ. Health 12: 73-87.(TET BR - acute, 0,3,6,9 mg/kg IP; salt; rat; SD; male & female, PND5; TC; **DR**, DEV; **BEHAV**, ANAT, TOXICOL; whole- or split-litter dosing models did not alter the effects of TET on mortality, brain weight or postweaning body weight but split-litter pups did have more persistent preweaning growth deficits and were more hyperactive, suggests dosing regimen is not a primary determinent of the development toxicity of TET

49. Ruppert, P. H., Dean, K. F. and Reiter, L. W. (1983). Developmental and behavioral toxicity following acute postnatal exposure of rat pups to trimethyltin. Neurobehav. Toxicol. Teratol. 5: 421-429. (TMT OH - acute, 0, 4, 5, 6 mg/kg IP; base; rat; LE; male & female, PND5; TC; **DEV**, MD, SN; **BEHAV**, ANAT, TOXICOL; body growth was retarded only during the preweaning period and organ weights were not affected but TMT caused permanent dose-related decreases in whole brain, hippocampus and olfactory bulb weights, as adults TMT treated animals were hyperactive and had alterations in the acoustic startle response)

50. Ruppert, P. H., Walsh, T. J., Reiter, L. W. and Dyer, R. S. (1982). Trimethyltin-induced hyperactivity: Time course and pattern. Neurobehav. Toxicol. Teratol. 4: 135-139. (TMT CL - acute, 0, 5, 6, 7 mg/kg OR; base; rat; LE, male, 60 d; TC; **CU**, SN; **BEHAV**, ANAT; an examination of the role of chemically induced hippocampal damage in the genesis of hyperactivity, clear hyperactivity was present by 4 days after dosing and still apparent at 32 days but only for the 7 mg/kg group, all doses caused hippocampal damage as indicated by a decrease in the length of the pyramidal cell line, hyperactivity was not related to the degree of hippocampal damage and may be due to damage in other brain areas)

51. Stanton, M. (199x). Neonatal exposure to triethyltin disrupts olfactory discrimination learning in preweanling rats. CLEARED MANUSCRIPT. (TET SUL - acute, 0, 3, 5 mg/kg IP; base; rat, LE, male & female, PND5, 10, 16; TC; **DEV**, CU; **BEHAV**, ANAT; olfactory discrimination deficits in TET-treated rats suggest an early impairment of associative learning and changes in nonassociative processes were not the cause; age of exposure and testing interacted to produce differential effects on learning, brain weight was decreased by 5 mg/kg TET on all days of exposure with the greatest decrease after PND5 TET; data suggests a dissociation of the behavioral, neural and somatic effects of TET)

52. Stanton, M. E., Jensen, K. F., Pickens, C. V. (199x). Neonatal exposure to trimethyltin disrupts spatial delayed alternation learning in preweanling rats. CLEARED MANUSCRIPT. (TMT OH - acute, 0, 6 mg/kg IP; base; rat, LE, male & female, PND10; No TC; **MD**, DEV, SN **BEHAV**, ANAT; during the preweaning period TMT-treated pups were unable to learn a discrete-trials delayed alternation T-maze task but could learn a simple position discrimination suggesting motor abnormalities were not a determinant of the deficient performance; spatial working memory deficits can be detected during the preweaning period and may be predictive of adult spatial working memory deficits; light microscopic examination showed loss of pyramidal cells and reduction in size of hippocampus after TMT).

53. Stine, K. E., Reiter, L. W. and Lemasters, J. J. (1988). Alkyltin inhibition of ATPase activities in tissue homogenates and subcellular fractions from adult and neonatal rats. Toxicol. Appl. Pharmacol. 94: 394-406. (TET BR - acute, 0, 6 mg/kg IP; TMT BR,rat, CD, male, 60 D; male & female, PND5; TC; **EOV BIOCHEM**; higher concentrations of TET are necessary to inhibit mitochondrial ATPase in adult brain homogenates than in neonatal brain or isolated brain mitochondria; the tin concentrations in the brain of adults after a neurotoxic dose of TET are insufficient to inhibit ATPase suggesting that inhibition of this enzyme is not a factor in adult TET neurotoxicity; in the neonate TET brain levels are high enough to inhibit ATPase and may interfere with mitochondrial function causing neuronal damage)

54. Veronesi, B. and Bondy, S. (1986). Triethyltin-induced neuronal damage in postnatally exposed rodents. Neurotoxicology, 7: 207-216. (TET - acute, 0, 6 mg/kg IP; salt; rat, LE, male, PND5; No TC; **DEV**; SN; **ANAT**, BIOCHEM; light microscopic examination at PND20 indicate TET altered Timm's, GFAP and acetylcholinesterase staining patterns in hippocampus and caused reduced whole brain and hippocampus weights as well as decreased cholinergic receptor binding in hippocampus; starved controls indicate CNS alterations are not a function of nutritional deficits; TET may interfere with development of neuronal as well as myelin elements)

55. Walsh, T. J., Miller, D. B. and Dyer, R. S. (1982). Trimethyltin, a selective limbic system neurotoxicant, impairs radial-arm maze performance. Neurobehav. Toxicol. Teratol. 4: 177-183. (TMT CL - acute, 0, 6 mg/kg OR; base; rat, LE, male, adult; TC; **MD**; SN; **BEHAV**, ANAT; rats trained in a radial-arm maze and then treated with TMT showed gradual development of hyperactivity and persistent deficits in accuracy suggesting CNS damage induced by TMT interferes with learning and memory processes; light microscopic examination and quantification indicated pyramidal cell line length was reduced but this indicator of hippocampal damage did not account for the behavioral changes)

56. Walsh, T. J., Gallagher, M., Bostock, E. and Dyer, R. S. (1982). Trimethyltin impairs retention of a passive avoidance task. Neurobehav. Toxicol. Teratol. 4: 163-167. (TMT CL - acute, 0, 5, 6, 7 mg/kg OR; base; rat, LE, male, 90 - 120 d & 250 - 300 g; No TC; **SN**; **BEHAV**; shorter step-through latencies and freezing durations during the retention test of a one-trial passive avoidance task, but no differences in footshock sensitivity, indicate rats treated with TMT have compromised learning and memory abilities)

*Primary research area addressed and primary method employed is in bold type.

ABBREVIATIONS: methods development (MD); environmental and organismic variables (EOV); developmental neurotoxicity (DEV); specific neurotoxicity (SN); dose-response and exposure scenarios (DR); conceptual understanding of neurotoxicity (CU); review (REV); time course (TC); oral (OR); intraperitoneal (IP); intravenous (IV); subcutaneous (SC); triethyltin (TET); trimethyltin (TMT); tributyltin (TBT); bromide (BR); hydroxide (OH); chloride (CL); oxide (oxide); sulfate (SUL); Long-Evans (LE); Sprague-Dawley (SD); gestational day (GD); postnatal day (PND); anatomical (ANAT); analytical (ANALY); behavior (BEHAV); biochemical (BIOCHEM); central nervous system (CNS); electrophysiology (ELEC); physiology (PHY); teratology (TERATOL); toxicology (TOXICOL); visual evoked response (VER); day (d); gram (g);