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Tetramethylenedisulfotetramine: pest control gone awry

Michael P. Shakarjian[1,2,3], **Marcela Laukova**[1], **Jana Velíšková**[2,4,5], **Patric K. Stanton**[2,5], **Diane E. Heck**[1], and **Libor Velíšek**[2,5,6]

[1]Department of Environmental Health Science, School of Health Sciences and Practice, Institute of Public Health, New York Medical College, Valhalla, New York

[2]Department of Cell Biology and Anatomy, New York Medical College, Valhalla, New York

[3]Department of Medicine, Division of Pulmonary and Critical Care Medicine, Rutgers-Robert Wood Johnson Medical School, Piscataway, New Jersey

[4]Department of Obstetrics and Gynecology, New York Medical College, Valhalla, New York

[5]Department of Neurology, New York Medical College, Valhalla, New York

[6]Department of Pediatrics, New York Medical College, Valhalla, New York

Abstract

Incidences of pesticide poisonings are a significant cause of morbidity and mortality worldwide. The seizure-inducing rodenticide tetramethylenedisulfotetramine is one of the most toxic of these agents. Although banned, it has been responsible for thousands of accidental, intentional, and mass poisonings in mainland China and elsewhere. An optimal regimen for treatment of poisoning has not been established. Its facile synthesis from easily obtained starting materials, extreme potency, and lack of odor, color, or taste identify it as a potential chemical threat agent. This review describes the toxicologic properties of this agent, more recent advances in our understanding of its properties, and recommendations for future research.

Keywords

tetramethylenedisulfotetramine; rodenticide; countermeasure; neuorotoxicity

Despite the significant benefits of pesticides in reducing infectious disease, agricultural losses, and food contamination, the risk of morbidity and mortality as a consequence of their production, storage, and use exists because they are inherently toxic, with limited, or in some cases, no selectivity. There are over 17,000 pesticide products marketed in the United States,[1] and their wide availability and variety makes it a challenging task to identify the source and treat victims of a pesticide exposure, especially outside of occupational settings. This task is magnified by the myriad of pesticides, prohibited, obsolete, or no longer in the U.S. market, that are still legitimately available elsewhere in the world. And finally, there are

Address for correspondence: Department of Environmental Health Science, School of Health Sciences and Practice, Institute of Public Health, New York Medical College, Vosburgh Pavilion, Rm 104, Valhalla, NY 10595. michael_shakarjian@nymc.edu.

Conflicts of interest

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"black market" pesticides that are banned in the United States and elsewhere but remain in production because of the ease of manufacture (high yield/cost ratio), profitability, and benefits (as doubtful as they may be) for the consumer.[2] The intensity of contemporary global travel and commerce raises the possibility of exposure to obscure pesticides within our domestic boundaries. Such was the case in New York City, where, in 2002, a 14-month-old child ingested a Chinese rodenticide powder her parents had deposited in the corners of their kitchen.[3] The rodenticide produced life-threatening and difficult-to-control convulsive status epilepticus, and was determined, months later, to contain tetramethylenedisulfotetramine (tetramine, TETS, TMDT; CAS# 80-12-6).[3]

Discovery of TMDT

TMDT (Fig. 1) is a synthetic polyhedral organic compound related to adamantane, first identified by Audrieth and colleagues,[4] after the initial synthesis by Wood and Battye.[5] Discovery of the toxicological effects of this agent occurred serendipitously, when material such as Crinex™, a rayon fabric manufactured by Farbenfabriken Bayer, was impregnated with sulfamide and formaldehyde as a fire retardant and supplied to factories.[6,7] Factory workers exposed to fabric aerosols experienced headache, nausea, vomiting, seizures accompanied by foaming at the mouth, and neurological and psychological disturbances.[6,7] No significant cardiac or vascular changes were observed. The impregnated Crinex fabric was identified as the offending material by mouse bioassay and process of elimination, demonstrating that the combination of formaldehyde and sulfamide were required for the manifestation of the seizure syndrome in mice.[6,7] Reaction of the impregnating materials yielded TMDT, which replicated the toxicity syndrome.[6] Lethality was observed after subcutaneous injection of 0.15–0.2 mg/kg in mice, with lethal doses at the same order of magnitude for guinea pigs, rabbits, cats, and dogs.[6]

Research by U.S. Forest Service

The effectiveness of TMDT against mice, five-fold more potent than any other pesticide of the time, prompted Bayer to patent TMDT and market the agent as a rodenticide in 1953.[8] Subsequently, the U.S. Forest Service took an interest in the agent.[9] The primary application was for the protection of tree seeds broadcast to reforest an area previously denuded by clearcutting or fire. TMDT was one of a handful of over 4500 candidates succeeding in a preliminary screen for seed protectants. Such agents are used, not to decimate the local rodent population by bait application, but rather to repel the rodents by coating the seeds with the poison and a distinct coloring, resulting in taste-aversion learning from pairing an adverse physiological reaction with the broadcast seeds. Extensive field application with TMDT was performed, with initially promising results. More TMDT-coated seeds survived and grew compared to untreated control seeds,[9] and translocation of the poison into the developing plant provided a margin of protection of the germinating seedling, evidenced by the toxicity of these seedlings to rodents during their first month of germination.[9] Later studies demonstrated that seedlings grown in soil treated with TMDT were toxic to hare for as long as 4 years.[10] However, early successes were not supported by the continued testing that followed,[11] and the extreme toxicity of TMDT was such that the original supplier soon

discontinued production of the chemical, primarily because of the hazards associated with its manufacture.[11] TMDT thus never became commercially available in the United States.[12]

Early investigations of TMDT actions

The above studies raised many questions regarding the toxicology of TMDT. Obtaining some material from the U.S Forest Service, Voss and colleagues performed a number of experiments to better understand its actions.[13] They found no major activity of TMDT on peripheral nerves or skeletal muscle; rather, convulsions were centrally controlled and dependent upon brainstem activity, as demonstrated in decerebrate mammalian models. TMDT also had no direct effect upon intestinal or bladder motility, or spleen or kidney size in spinal dogs, though it did increase respiratory rate.[13] Obvious concerns about accidental exposure prompted the group to test various agents in mice for their ability to counteract the seizure-producing actions of TMDT.[14] Pretreatment with sodium bromide or long-acting barbiturates increased latency and reduced the severity of TMDT-induced seizures, improving survival over a 5-day observation period. Conversely, urethane, chloral hydrate, sodium pentobarbital, and trimethadione were unable to offer satisfactory protection in their hands. The authors attributed these results to the shorter-acting nature of these agents, citing the slow elimination of TMDT in mice.[14]

Locus of TMDT action

Elucidation of the molecular sites of action of TMDT would have to wait until the 1970s, a better understanding of amino acid neurotransmitters, and the age of ligand–receptor binding. Curtis and Johnston[15] predicted that TMDT, on the basis of experimental observations and similarities in action to bicuculline and picrotoxinin, was likely an antagonist of GABA activity. In rat superior cervical ganglion neurons, Bowery *et al.*[16] found that TMDT concentration-dependently antagonized the depolarizing actions of GABA, while not affecting the actions of the cholinomimetic agent carbachol. This inhibition was reversed upon washing the preparation. Furthermore, in distinction to methyl-bicuculline, TMDT reduced the maximal response to GABA, suggesting a noncompetititive mechanism of inhibition. The investigators suggested that TMDT might occlude the GABA-operated ionophore to produce its effect. A subsequent report by the same group[17] demonstrated that the actions of TMDT (as well as picrotoxin (PTX), bicuculline, and isopropylbicyclophospate) in the superior cervical ganglion preparation could be reversed by pentobarbital. In screening bi- and tricyclic compounds, Casida *et al.* confirmed the seizurogenic actions of TMDT in mouse and frog models. While, in mice, these seizures were inhibited by phenobarbital, pretreatment with the mixed-function oxidase inhibitor piperonyl butoxide had no effect, suggesting that metabolic activation is not required for TMDT action.[18] Collins *et al.*[19] found that intravenous administration of TMDT could antagonize the actions of microiontophoretically applied GABA, as well as of glycine, in the cuneate nucleus of urethane-anesthetized rats. Three compounds structurally related to TMDT were without effect in this preparation. Large[20] corroborated these findings in a crustacean model (Hermit crab neuromuscular junction), demonstrating a dose-dependent, noncompetitive, and reversible action of TMDT that was similar to the effects of PTX in this model. Electrographic seizures were noted subsequent to either intravenous administration

or direct cerebrocortical application of TMDT in pentobarbital and urethane-anesthetized rats, respectively.[21,22]

A major turning point arrived in the understanding of GABAergic pharmacology with the advent of a radioligand-binding method for detecting binding sites. Using ^{3}H-dihydropicrotoxinin as the radioligand, Ticku and colleagues[23] demonstrated a reversible and saturable binding site in rat brain, distinct from but tracking with the GABA binding site, and to which naturally occurring antagonists of GABA activity, picrotoxinin and tutin, avidly bound. Further studies revealed that cage convulsants like bicyclophosphates and TMDT could displace ^{3}H-dihydropicrotoxinin specifically and with high affinity,[24] suggesting that their seizurogenic effects are due to interaction with GABA receptor ionophore complex.

New, more high-affinity radioligands with lower nonspecific binding now exist, allowing for improved accuracy in estimating binding constants. The $GABA_A$ receptor chloride ionophore has a pentameric structure, analogous to the nicotinic acetylcholine receptor, and comprising, at a minimum, α, β, and γ subunits (as well as less abundant δ, ε, π, θ, and ρ subunits), over which many variants exist, allowing for a broad variation of $GABA_A$ receptor subunit compositions with functional differences in mammalian brain. The most common variant in the adult brain is formed by 2xα1, 2xβ2, and 1xγ2.[25] Using [^{3}H]ethynylbicycloorthobenzoate (^{3}H-EBOB) as a radioligand and recombinant $GABA_A$ receptors, Ratra *et al.*[26] demonstrated differences between the pharmacological behavior of TMDT and picrotoxinin. The two ligands displayed similar affinities for $GABA_A$ receptors in human cortical membranes and α1β3γ2 subunit–containing recombinant receptors and similar potencies for inhibition of GABA-stimulated chloride flux from rat cerebral cortex vesicles.[27] However, while picrotoxinin displayed the highest affinity for recombinant $GABA_A$ pentameric receptors formed from the β3 subunit, TMDT was unable to displace ^{3}H-EBOB from this receptor.[26] This result clearly shows that, despite their pharmacological similarities, distinct differences exist in the affinities of these two agents for some variants of $GABA_A$ receptors. Indeed, Palmer and Casida[28] discerned two groups of $GABA_A$ receptor antagonists, type A (picrotoxin and cage convulsants with large substituents or a more extended structure) and type B (more compact cage convulsants, including TMDT), with different binding properties and species selectivities. Type A compounds rely upon hydrophobic interactions with $GABA_A$ receptor amino acid residues and are more likely to be potent insecticides, whereas type B compounds, which exhibit some polar interactions, are more toxic in vertebrate models. The physicochemical properties of TMDT differ greatly from most of the known ligands for the $GABA_A$ receptor pore, and this compound appears to rely almost exclusively upon polar interactions, resulting in a 300- to 500-fold selective toxicity for mammals over insects.[29] It is currently unknown whether the unique binding properties of TMDT provide it with selectivity for particular brain regions or $GABA_A$ receptor variants in the mammalian brain compared to convulsants like picrotoxin, nor is it known to what extent its physicochemical properties affect the ability to reverse its toxic actions.

TMDT becomes public health concern

Chemical poisonings in mainland China constitute a serious public health issue in which pesticide exposures (herbicides, insecticides, rodenticides) play prominent roles.[30,31] Surveys from several cities and provinces indicate that nonoccupational, rather than occupational, pesticide poisoning accounts for the great majority of reported incidents.[32–36] TMDT is one of the most toxic and deadly pesticides available in China.[34,35,37] A survey of TMDT intoxication in Chinese journals yielded over 14,000 poisonings with 932 deaths between January 1991 and December 2010.[38] Although efforts have been made to restrict the availability of this agent, it remains responsible for numerous poisonings and deaths, including occasional high-profile reports of mass casualties. Recent examples include two incidents where children were specifically targeted in efforts by one kindergarten to tarnish the reputation of a competing one. In Hebei Province, two young girls consumed TMDT-laced yogurt drink prepared by the head teacher of a rival institution,[39] and in Yunnan Province, a rival school's manager poisoned the water supply of one school, leading to hospitalization of 76 children and killing two.[40] Incidents such as these, and the unique characteristics of TMDT, raise concerns regarding its utility as a chemical agent of terror. Indeed, TMDT possesses key features of a terrorist chemical weapon, including facile synthesis from easily obtained starting materials; stability after preparation; high potency, with an estimated human lethal dose of 0.1 mg/kg; lack of color or odor; miscibility in food and drink; and persistent action after ingestion.[41]

Renewed interest in TMDT research

In light of these features, recent investigations have focused on better understanding the actions of TMDT and identifying treatment modalities with the potential to both save lives and reduce long-term morbidity of exposed individuals.

Various routes of exposure to TMDT have been examined in rodents. Mice administered 0.1–0.5 mg/kg TMDT intraperitoneally (IP) exhibited a consistent pattern of behaviors.[42,43] The initial behavioral change, occurring within the first 3 min, was a freezing behavior and cessation of locomotor activity. This was followed by twitches of the body and an erect and upright (Straub) tail, first signs of convulsive activity. Multiple twitches gave way to unilateral or bilateral clonic seizures consisting of forelimb clonus with rearing and righting reflex preserved. One or several clonic seizures occurred, after which a tonic–clonic seizure evolved. This consisted of a wild run, loss of righting reflex, tonic flexion, and extension of forelimbs and hindlimbs, followed by clonic movements of all limbs. If this seizure did not result in lethality, subjects recovered in several minutes. The animal could experience repeated tonic–clonic seizures before it eventually succumbed to or escaped the lethal consequences of this agent. At higher doses, a more rapid progression of the syndrome occurred, associated with fewer clonic and tonic–clonic seizures and a higher probability of death. As expected, oral dosing was reported to require higher dose equivalents to produce the key symptoms of poisoning.[43] Interestingly, a comparison of the actions of TMDT and PTX by two different routes reveals that TMDT is 5-fold more potent than PTX in precipitating seizures after IV administration, whereas, using the same measures, PTX is more potent than TMDT after intraventricular administration of the agents.[43] These results

suggest that a greater rate of disposition and/or slower rate of partitioning into the brain by PTX could explain the relative activities of these two agents as a function of administration route.[44]

Electrocortical changes

Accompanying TMDT-induced seizures are changes in EEG patterns in rodents that are typical and reflect the phenotype of myoclonic jerks, clonic seizures, and tonic–clonic seizures. As indicated above, Sobotka and Safanda[21] and Dray[22] provided descriptions of TMDT-induced alterations in cortical EEG in anesthetized rats, observing high amplitude spiking upon IV infusion or direct cortical application of the substance. We have recently documented the relation between electrocorticogram and seizure phenotype in unanesthetized, freely moving mice after exposure to TMDT alone or in combination with potential countermeasures.[42] Low-amplitude cortical EEG activity in the naive animal converted to synchronized, high-amplitude discharges occurring within 2 min of administration of TMDT (0.4 mg/kg, IP). Polyspike and spike and wave activity followed (associated with immobility/freezing) and preceded clonic motor seizure. This data suggests that the onset of TMDT-induced seizures is likely within the cortical network.[42] Finally, spike and wave discharges of lower amplitude occurred later, indicative of a tonic–clonic seizure, and following several seconds after the physical signs were observed in subjects. The delay in cortical EEG activity relative to observation of the seizure signs, together with the fact that TMDT-induced seizures occur in decerebrate animals, suggest that the origin of tonic–clonic seizures is within brain stem structures.[13] Human clinical cases have reported similar paroxysmal, high-amplitude unilateral and bilateral epileptic activity with generalized spike and slow wave discharges.[38,45,46]

Examination of potential countermeasures

In vitro and rapid-screening tools

Methods to measure GABA receptor–operated Cl^- channel activity have included use of $^{36}Cl^-$ as a tracer to ascertain Cl^- flux through the ion channel,[47,48] and/or by monitoring GABA-dependent intracellular acidification with a pH-sensitive fluorescent dye as a surrogate for Cl^- entry.[49] In contrast, monitoring changes in neuronal excitability downstream from the $GABA_A$ receptor complex, such as by measuring Ca^{2+} influx as a consequence of excitation, allows one to test agents having a variety of mechanisms of action against consequences of $GABA_A$ receptor blockade. Cao *et al.* adopted this method for the purposes of screening compounds as potential countermeasures of TMDT poisoning.[50] Dissociated hippocampal cell cultures from newborn mice, grown for 13–17 days *in vitro,* exhibit spontaneous Ca^{2+} oscillations, as measured by Ca^{2+}-sensitive fluorescent dye.[50] These oscillations are sensitive to the *N*-Methyl-D-aspartate (NMDA) open channel blocker MK-801, but not to the voltage-dependent calcium channel blocker nifedipine, suggesting that NMDA receptor–operated channels, and not L-type Ca^{2+} channels, are involved in TMDT-induced spontaneous activity. TMDT, bicuculline, and PTX all disrupted these oscillations in a similar fashion. The $GABA_A$ channel positive modulators diazepam (DZP) and pregnanolone reversed TMDT actions in a concentration-dependent

manner when applied to cultures individually and produced a cumulative effect when added as a combination. An analogous screening method, using cerebral cortical cell cultures from embryonic day 18 rats, is also being applied to identify potential treatments for TMDT exposure.[51] A preliminary result from this screen indicates that the NMDA receptor antagonists MK-801 and ketamine display a more persistent antagonistic effect upon TMDT action than that seen with DZP.[51]

A potential *in vivo* rapid-screening tool under exploration involves the use of larval zebrafish (*Danio rerio*).[52] Unlike insects, $GABA_A$ receptors of teleost fish are heteropentamers more akin to receptors found in mammals.[53–55] The organism's small size, responsiveness to a variety of seizurogenic agents, and the availability of automated methods of assessment make them a potentially useful and relevant model to screen for TMDT countermeasures.

In vivo mouse models

In humans, a whole battery of anticonvulsant agents have been administered to attempt to control generalized seizures produced by TMDT exposure. In addition to gastric lavage to remove TMDT from the stomach, pharmacotherapy is necessary to control actions of the poison already absorbed. The list of agents includes benzodiazepines, barbiturates, diphenylhydantoin, valproate, carbamazepine, topiramate, and ketamine, with polytherapy often needed for complete seizure control.[41,45] There are also reports that pyridoxine and dimercaptopropanesulfonate can be useful as treatments.[41,45]

Given the common use of benzodiazepines in the treatment of status epilepticus[56,57] and our previous work with NMDA receptor antagonists as antiseizure agents,[58–61] we examined the ability of diazepam, ketamine, and MK-801, administered intraperitoneally, to control seizures and reduce lethality in mice exposed to TMDT. A 15-min pretreatment with these agents before exposure to TMDT and posttreatment immediately following the first TMDT-induced clonic seizure exhibited roughly equivalent dose-dependent efficacy in controlling seizures and preventing short-term lethality.[42] Lower doses of ketamine (35 mg/kg) and MK-801 (0.5 mg/kg) were associated with potentiation of clonic seizures, as previously reported for their actions in other seizure models,[60,61] but had no effect on tonic–clonic seizures. A low dose of DZP (1 mg/kg) had no significant effects on seizure incidence and lethality. Higher doses of these agents all provided good protection of the subjects, however. Compared to 1 mg/kg MK-801, 5 mg/kg DZP was unable to eliminate the abnormal electrographic activity produced by TMDT, and these animals were more likely to succumb to TMDT's lethal effects hours later (Fig. 2). We subsequently examined combinations of DZP and MK-801 in order to take advantage of the most positive aspects of each of these agents as treatments. Simultaneous administration of DZP and MK-801 produced synergistic protection against tonic–clonic seizures and 24-h lethality in TMDT-exposed mice, as determined by isobolographic analysis (Fig. 3).[62,63] In contrast, clonic seizures remained poorly controlled. A change to a sequential therapeutic regimen where MK-801 was administered 10 min after DZP treatment further improved the outcome, with good control of clonic seizures and reduction of overall severity of the syndrome relative to the simultaneous administration of the agents (Fig. 4). These studies suggest that sequential

administration of benzodiazepine–NMDA receptor antagonist regimens may be a more effective clinical therapeutic regimen to counteract TMDT exposures.

Choosing agents based upon *in vitro* investigations,[50] Bruun *et al.*[64] examined the ability of DZP, allopregnanolone, and their combinations to antagonize seizures and lethality produced by TMDT exposure in rodents. They found, in both pre- (10 min before TMDT administration) and posttreatment (2 min after the second clonic seizure) paradigms, that a subthreshold, or a partially effective dose of each agent (0.1 mg/kg IP) when administered simultaneously, achieved an improved 24-h survival. Also observed in the 72 h following TMDT exposure were astrogliosis, as evidenced by GFAP immunoreactivity, and decreases in microglial activation, in the cerebral cortices and hippocampi of mice treated with the two-agent combination as compared to high-dose DZP (5 mg/kg) treatment. While both DZP and allopregnanolone have binding sites on $GABA_A$ receptor ionophore complexes, the identity of complexes affected may differ (i.e., synaptic versus extrasynaptic), and, when located on the same complex, they can allosterically influence each other's binding, providing a basis for positive interaction in promoting $GABA_A$ receptor complex function.

Inceoglu and colleagues[65] reported that inhibitors of soluble epoxide hydrolase, an enzyme that degrades epoxyeicosatrienoic acids, can modify the actions of $GABA_A$ complex antagonists pentylenetetrazole and PTX, increasing latency and preventing tonic seizure activity. These agents had no ameliorating effects upon seizures produced by either maximal electroshock or the voltage-gated K^+ channel blocker 4-aminopyridine. More recently,[66] this group found that pretreatment with the soluble epoxide hydrolase inhibitor TUPS could prevent TMDT-induced tonic seizures and lethality. Unfortunately, this agent was not active in a postexposure paradigm, but the results of these studies suggest that endogenous physiological antiseizure molecules may be generated upon $GABA_A$ receptor blockade.

Confounding data

Given the reports of protracted neurological effects of TMDT in humans, including in those who did not experience severe seizures upon exposure,[45,67] attempts at finding experimental evidence for chronic neuropathological effects in animal models have been surprisingly unproductive.

Lack of kindling effect

One common characteristic of $GABA_A$ receptor blockers is their ability to produce chemical kindling,[68] a long-lasting decrease in seizure threshold after repeated administration of subconvulsant doses of a seizurogenic agent, considered to be a model of the epileptogenic process. In contrast to pentylenetetrazole (PTZ), attempts to demonstrate such a phenomenon by using TMDT have not been successful.[43] This may have been because TMDT was unable to increase activation of anatomic pathways implicated in the phenomenon. Conversely, it might be that the characteristics of TMDT in regard to potency and persistence in the body make it more difficult to discover the dosing paradigm necessary in order to elicit a kindling response.

Lack of persistent behavioral pathology in rodent models

In an effort to reveal long-term changes in behavior, Flannery *et al.*[69] exposed three cohorts of adult male Swiss mice to 0.15 mg/kg IP TMDT, followed by rescue after the second clonic seizure by administration of 5 mg/kg IP DZP. Surviving animals (75%) were then tested in a battery of behavioral tests in 1-week, 1-month, or 2-month postexposure cohorts. While displaying a slight increase in vertical locomotor activity in the 2-month cohort, TMDT-exposed mice showed no alterations in measures of anxiety (elevated plus maze and light–dark transition tests), depressive-like behaviors (tail suspension and Porsolt forced swim tests), or memory (novel object recognition). The authors concluded that, while species differences might explain why humans and not mice display behavioral impairment to TMDT exposure, they considered it more likely that persistent status epilepticus, a feature lacking in their model, may be required to produce these changes. Nevertheless, there are other behavioral tests (i.e., measuring spatial behavioral learning, contextual fear conditioning, or active avoidance) that may be employed to reveal TMDT-induced alterations.

Lack of delayed neuronal cell death

Brains of mice displaying clonic seizures from 0.11 to 0.2 mg/kg TMDT IP were examined for signs of histopathology (cortex, hippocampus, cerebellum) 4 h to 7 days postexposure.[43] No evidence of neuronal death or cell loss was observed during this time frame, as determined by Fluoro-Jade B or by comparative staining of adjacent sections by H&E or DAPI. However, sections displayed a consistent reactive gliosis considered indicative of neuroinflammation. This lack of specific neuronal involvement may be due to absence of tonic–clonic seizures in the subjects. The difficulty, however, is in metering the dose of TMDT in order to produce this type of seizure while also ensuring survival of the subject. There is a need for more detailed examination of neuronal survival and inflammatory processes to determine what animal model correlates exist to the behavioral and neurodevelopmental consequences of TMDT exposure in humans.

Future directions

From information gleaned over the last 70 years, it is clear that TMDT shares many toxicological characteristics with the various families of $GABA_A$ receptor blockers, such as the naturally occurring sesquiterpene lactones and the synthetic bicyclophophosphates and organochlorine insecticides, yet also displays unique properties, due to its potency, physicochemical properties, and those characteristics that place it in the category of a chemical threat agent.[70]

There is significant need for a clearer understanding of the toxicokinetics of TMDT, at least from the most common route of exposure, oral. Very little is known regarding the absorption, distribution, metabolism, and excretion of TMDT. Reports from China, using various means to extract TMDT from blood as an important mode of detoxification (hemoperfusion and hemofiltration), found blood levels to drop initially and then rebound.[45,71] This suggests that TMDT persists in certain tissues in primarily unchanged form for long periods of time. Radwan and Dodge[72] administered ^{14}C-TMDT orally to mice

and collected radioactivity at various time points up to 96 h. They documented rapid but apparently incomplete absorption of TMDT from the gastrointestinal tract and efficient distribution to the major organs. A metabolite, more hydrophobic than the parent compound, was visualized from extracts of certain tissues, migrating with a contaminant of synthesis (possibly HEXS). Approximately 98% of recoverable ^{14}C was eliminated by 72 h. Elimination was mainly via the feces, estimated at three times that of urinary excretion; some radioactivity was also released as $^{14}CO_2$ in the expired air. In a more recent, contrasting report,[73] rabbits were exposed to TMDT via intravenous and oral routes, and samples analyzed by GC with nitrogen–phosphorous detection. Half-lives of elimination were 57 h and 262 h for intravenous and oral routes, respectively. Elimination was primarily via the urine, with less than 20% excreted in the bile. Administration of activated charcoal immediately after TMDT exposure shortened the oral elimination half-life by 55%. These reports underscore the necessity for additional toxicokinetic studies in order to clearly understand TMDT disposition.

Also critically important will be a better understanding of age- and sex-related differences in susceptibility to TMDT. Reports from China indicate that males and females are equally likely to be victims of pesticide poisonings. Furthermore, one study suggests that roughly one-third of all victims of pesticide poisonings in China are young children from 0 to 4 years of age,[30] and there are ample instances where children have been targeted in intentional poisoning attempts with TMDT. Preliminary animal studies[74,75] indicate that postnatal day 15 rats are much more sensitive to TMDT as compared to adults or even 25-day-old animals. Response to treatments also appears to differ as a function of age.[74]

Much needs to be learned with regard to the cellular and molecular events initiated by TMDT and their relevance to short- and long-term pathology. Zolkowska and colleagues[43] first described reactive gliosis produced by TMDT exposure. It is currently unclear whether these changes constitute injurious events, attempts by homeostatic mechanisms toward neuroprotection, or both. A more detailed examination of glial proliferation and inflammatory markers may reveal the significance of the current findings. Furthermore, little has been identified regarding molecular events precipitated by TMDT exposure, not only by glia, but also by neuronal networks. The example of changes in epoxy fatty acid levels is certainly intriguing.[65] A further understanding of changes such as these will be necessary in devising new treatments that move beyond immediate survival to address long-term consequences.

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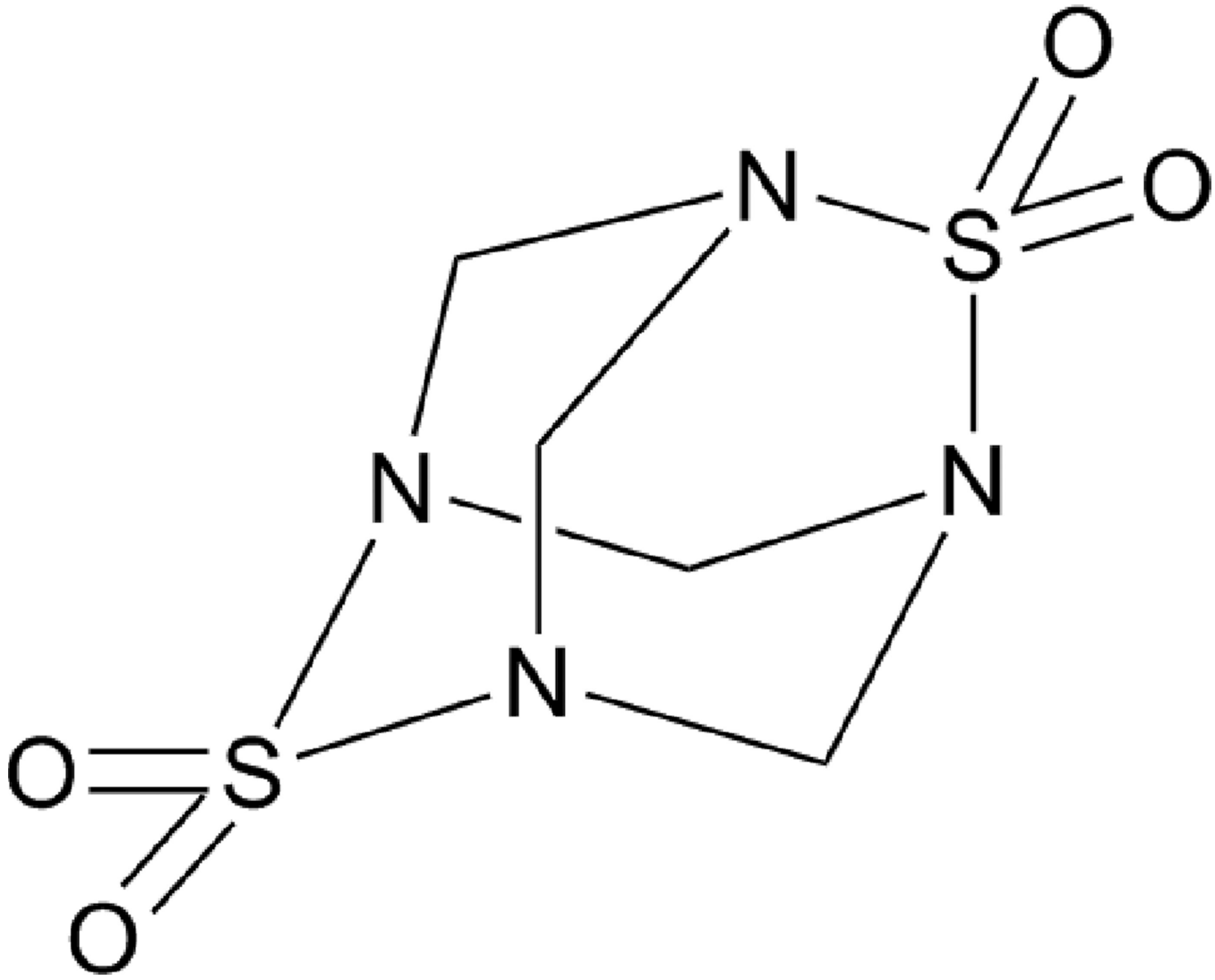

Figure 1.
Chemical structure of tetramethylenedisulfotetramine, CAS# 80-12-6, IUPAC name 2,6-Dithia-1,3,5,7-tetraaza-tricyclo[3.3.1.1^{3,7}]decane 2,2,6,6-tetraoxide.

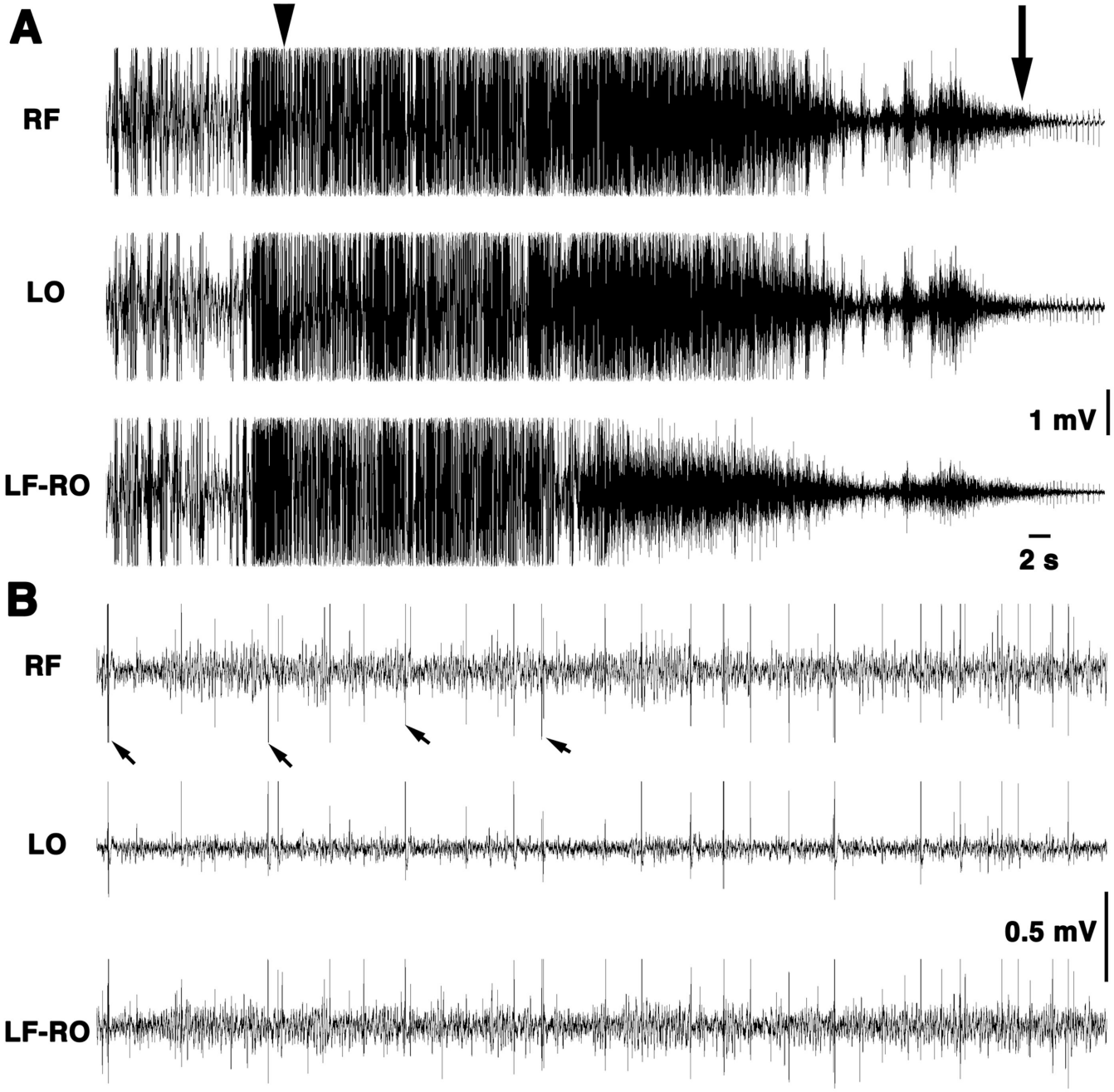

Figure 2.

Long-term EEG/video recordings reveal delayed seizures and death after TMDT injection with DZP posttreatment. (A) This mouse was injected with 0.4 mg/kg TMDT and, with the occurrence of the first clonic seizure, received 5 mg/kg of DZP IP. Both EEG and motor activity were continuously recorded from before the TMDT injection through death approximately 9 h after TMDT administration. EEG recordings document ictal activity of a clonic seizure transitioning into a tonic–clonic seizure (arrowhead), which ended lethally. Motor convulsions ceased to be present at the arrow. Please note high-amplitude discharges associated with tonic–clonic seizure (actually preceding the seizure by several seconds).

Decreasing amplitude of EEG discharges was associated with diminishing motor activity ending lethally. (All calibrations 1 mV; time mark 2 s). (B) This mouse was also injected with 0.4 mg/ kg TMDT and, with the occurrence of the first clonic seizure, received 1 mg/kg of MK-801 IP. The EEG shown here was recorded at the corresponding time after TMDT injection as in Fig. 2A. However, the EEG of the mouse posttreated with MK-801 shows only individual discrete interictal discharges (some marked with arrows) without any behavioral correlates. The mouse survived 24 h of recording. (All calibrations 0.5 mV; time mark 2 s). From Ref. 42.

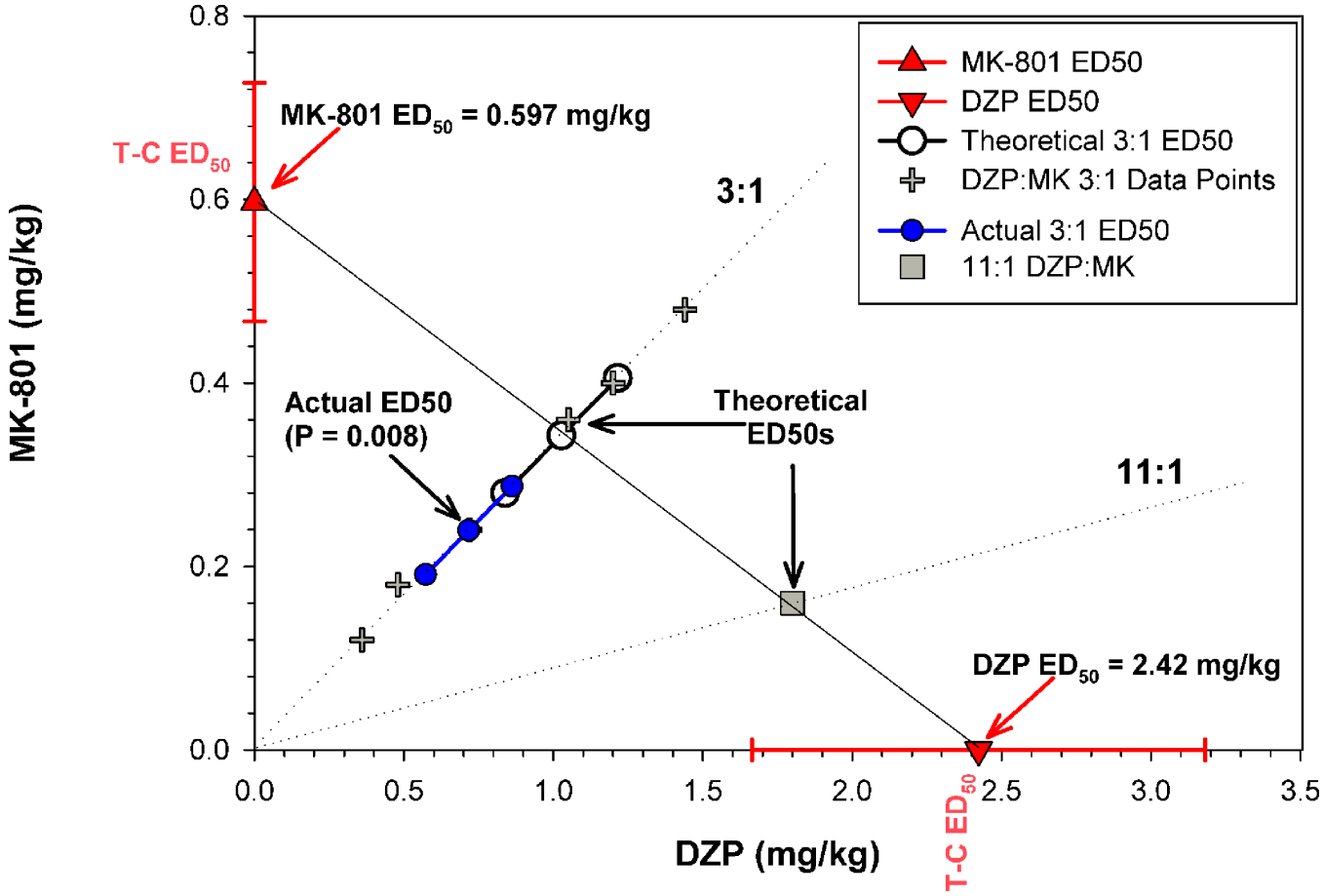

Figure 3.

Isobologram for the interaction of DZP and MK-801 in suppressing TMDT-induced tonic–clonic seizures. ED_{50}s ± 95% CI of DZP (downwards triangle) and MK-801 (upwards triangle) administered singly were plotted on the *x* and *y* axes, respectively. The line of additivity connects these ED_{50}s. Open circles: theoretical ED_{50} ± 95% CI for inhibition of tonic–clonic seizures by DZP:MK-801 3:1. Closed circles: actual ED_{50} ± 95% CI of the DZP:MK-801 3:1 combination. This dose was significantly below the theoretical point of additivity ($P < 0.006$). Dotted lines depict the 3:1 and 11:1 DZP:MK-801 fixed ratios. Treatment with the predicted ED_{50} dose for the DZP:MK-801 11:1 combination yielded greater inhibitory activity than expected, suggesting that synergy observed by these two agents is not isolated to their 3:1 combination. From Ref. 62.

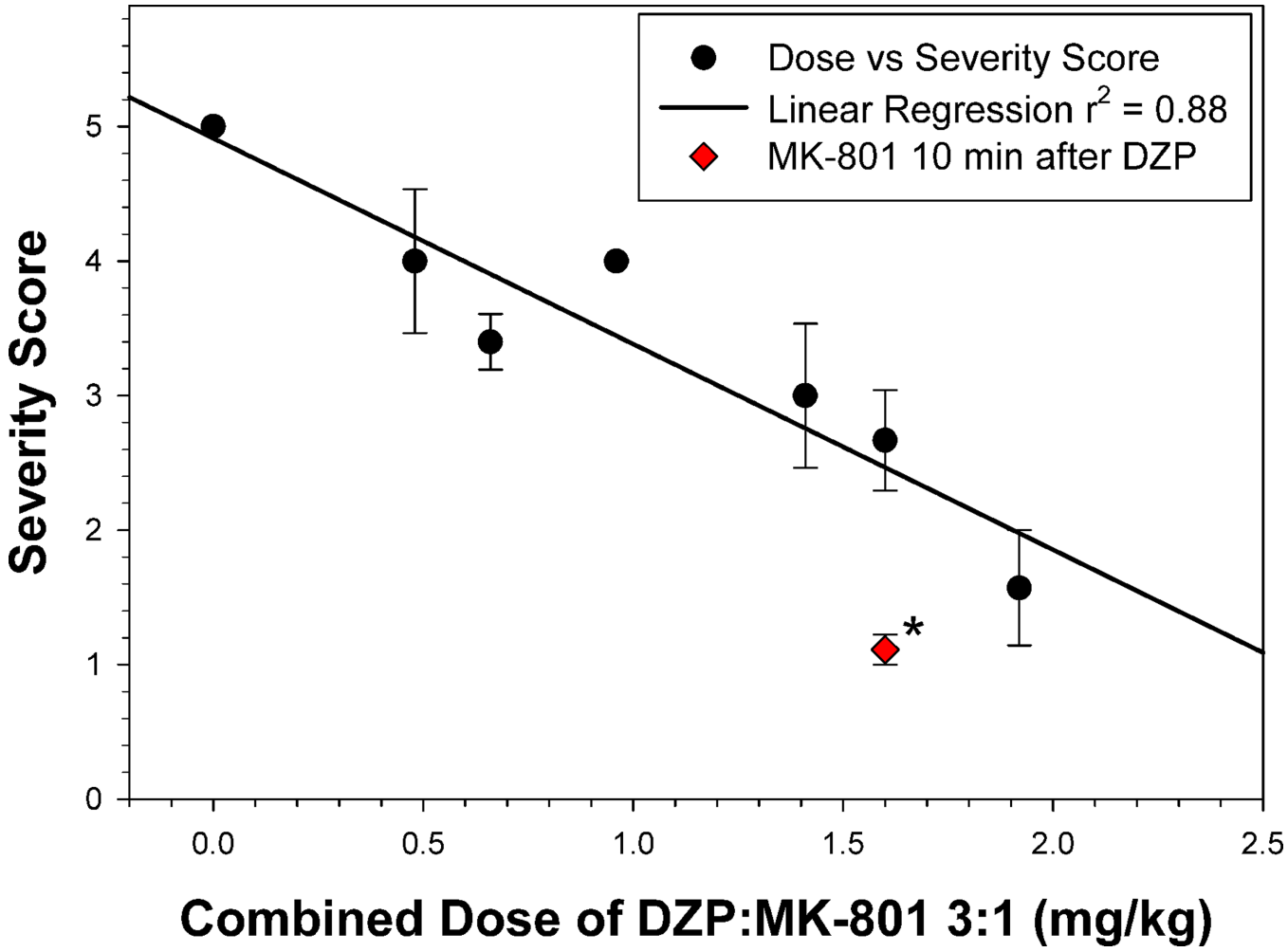

Figure 4.

Reduction in severity of TMDT symptoms by combined DZP and MK-801 treatment. The figure depicts severity score as a function of dose of the DZP:MK-801 combination treatment. Circles: DZP:MK-801 3:1 fixed ratio doses; solid line: linear regression of these points ($R^2 = 0.88$). Diamond: a 3:1 dose (DZP:MK-801 1.2:0.4 mg/kg), where MK-801 was administered 10 min after DZP administration. This latter regimen provided a significantly improved severity score relative to simultaneous administration of DZP and MK-801 ($P = 0.0013$, Mann–Whitney U-test). From Ref. 62.