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"Evaluación del mecanismo de alteración de la función espermática por exposición al larvicida organofosforado temefos"

Tesis que presenta:

QFB Juan Pablo Martínez Mercado

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Directora de tesis:

Dra. María Betzabet Quintanilla Vega

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Este trabajo se realizó en el Departamento de Toxicología del Centro de Investigación y de Estudios Avanzados del IPN bajo la tutoría de la Dra. María Betzabet Quintanilla Vega. Debido a la situación de la pandemia de marzo 2020 a agosto 2021 que no permitió actividades presenciales en el Cinvestav, esta tesis consiste en una revisión bibliográfica del tema de esta que se enviará a la revista *Environmental Pollution* para su publicación.

RESUMEN

El temefos (O,O,O',O′-tetrametil O,O′-difenilen-p-tiolfosforotioato) es un larvicida perteneciente a la familia de plaguicidas organofosforados (OP), utilizado para el control de diferentes vectores de enfermedades, como dengue, zika, chikungunya y dracunculiasis. La Organización Mundial de la Salud (OMS) lo clasifica en clase III: levemente peligroso, con un LOAEL (nivel de efecto adverso más bajo observable) de 100 mg/Kg/día hasta por 44 días en ratas, estableciendo que tiene muy baja toxicidad para el ser humano. El objetivo de esta revisión fue discutir la información disponible publicada sobre la cinética y toxicidad del temefos en los mamíferos.

La gran mayoría de los estudios encontrados sobre este plaguicida están relacionados con su uso, así como la resistencia, susceptibilidad y toxicidad producida en las larvas de algunos mosquitos. Solo 24 artículos estuvieron relacionados con su cinética o toxicidad en mamíferos. El temefos tiene una absorción rápida por vía oral, presentando mayor distribución y afinidad en tejidos con alto contenido lípido. Al momento, se han logrado identificar 11 metabolitos de este compuesto, entre los cuales se encuentran oxones, dioxones, compuestos monohidrolizados y derivados fenólicos, producidos por reacciones de S-oxidación, desulfuración oxidativa y desfosforilación. Aún no se han identificado cuales enzimas son las que participan, pero se sugiere que algunas monooxigenasas de flavina (FMO) y citocromos P450 (CYP) son las responsables. Es necesario completar el metabolismo de este plaguicida, tanto de la fase I como de fase II.

Entre los diversos efectos tóxicos descritos para el temefos está la capacidad de inhibir la enzima acetilcolinesterasa (AChE), incluso a concentraciones similares a la LOAEL. Sus metabolitos más oxidados son los que tienen mayor capacidad de inhibir esta enzima. También se encontró que el temefos tiene la capacidad de producir daño en el ADN por exposición ocupacional y a concentraciones empleadas para el control de vectores; así como también la formación de micronúcleos a concentraciones menores a la LOAEL. Adicionalmente, el temefos produce efectos tóxicos en la reproducción masculina, entre ellos, disminución de la calidad espermática, capacitación espermática y reacción acrosomal que llevan a una reducción del potencial de fertilización de los espermatozoides a concentraciones consideradas seguras por la OMS. De manera interesante, entre los órganos blanco de este larvicida también se encuentra el hígado, en el cual produce necrosis y disminución en su peso a la dosis LOAEL propuesta por la OMS, así como alteraciones en algunas enzimas hepáticas y en la homeostasis de los lípidos. Todos estos datos muestran la toxicidad del temefos en los mamíferos, aún a concentraciones que son consideradas seguras por algunas agencias, lo que sugiere que la seguridad de este plaguicida para los mamíferos debe reconsiderarse y pone una alerta sobre su uso.

ABSTRACT

Temephos (O,O,O',O′-tetramethyl O,O′-thiodi-*p*-phenylene bis (phosphorothionate)) is a larvicide belonging to the family of organophosphate (OP) pesticides, used for the control of different vectors of diseases, such as dengue, zika, chikungunya, and dracunculiasis. The World Health Organization (WHO) classifies temephos in class III: slightly dangerous, with a LOAEL (lowest observable adverse effect level) of 100 mg/Kg/day for up to 44 days in rats, establishing that it has very low toxicity for mammals. The aim of this review was to discuss the available published information on the kinetics and toxicity of temephos in mammals.

Most studies about this pesticide are related to its use, as well as the resistance, susceptibility, and toxicity produced in the larvae of some mosquitoes. Only 24 articles were related to its kinetics and toxicity in mammals. Temephos is rapidly absorbed following an oral route and it is mostly distributed to tissues with high lipid content. To the date, 11 metabolites of temephos have been identified including oxons, dioxons, monohydrolyzed compounds, and phenolic derivatives, which are produced by S-oxidation, oxidative desulfurization and dephosphorylation reactions. The enzymes involved have not been identified yet, however, some flavin monooxygenases (FMO) and cytochromes P450 (CYP) may be potentially involved. Thus, future studies aimed to complete the metabolism of this pesticide for both phase I and phase II are warranted.

The toxic effects of temephos we described in the review include the inhibition of the enzyme acetylcholinesterase (AChE), even at doses similar to the LOAEL. Its most oxidized metabolites are those having the greatest capacity to inhibit this enzyme. It was also found that temephos has the ability to produce DNA damage in occupational exposure and at concentrations used for vector control, as well as the formation of micronuclei at concentrations lower than the LOAEL. In addition, temephos produces toxic effects on male reproduction, including decreased sperm quality, sperm capacitation, and acrosomal reaction that lead to reduced fertilization potential of spermatozoa at concentrations considered safe by the WHO. Interestingly, the liver is also a target organ, in which temephos produces necrosis and decreased weight at the LOAEL dose proposed by the WHO, as well as alterations in some liver enzymes and lipid homeostasis. All these data together demonstrate the toxicity of temephos in mammals, even at concentrations that are considered safe by some agencies. Thus, safety of temephos must be reconsidered and its use should be alerted about its potential toxicity.

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ABREVIATURAS

AChE	Acetilcolinesterasa
CYP	Citocromos P450
DL50	Dosis letal media
EPA	Agencia de Protección Ambiental
FMO	Monooxigenasas de flavina
LOAEL	Nivel más bajo de efectos adversos observados
OP	Organofosforados
OMS	Organización Mundial de la Salud

1. INTRODUCCIÓN

Se considera plaguicida a cualquier sustancia destinada a prevenir, destruir, atraer, repeler o combatir cualquier plaga de especies indeseadas de plantas o animales. Los plaguicidas emergieron entre 1930 y 1940 como resultado de investigaciones que tenían como objetivo la eliminación y el control de insectos. Son diversas las clasificaciones que se le pueden dar a plaguicidas, pero las más comunes son con base en su estructura química, objetivo blanco, toxicidad aguda, vida media, usos, entre otros. La Organización Mundial de la Salud (OMS) en el 2009 y la Agencia de Protección Ambiental (EPA) en 2001 establecieron una clasificación basada en la toxicidad aguda y la peligrosidad de los plaguicidas. La toxicidad se determinó en base a la dosis letal media (DL_{50}), es decir, la dosis que mata al 50% de la población expuesta (Tabla i).

Tabla i. Clasificación de los plaguicidas de acuerdo con su toxicidad.

Toxicidad aguda de los plaguicidas			
Clasificación OMS		**Clasificación EPA**	
Ia	Extremadamente peligroso	**I**	Altamente tóxico
Ib	Altamente peligroso		
II	Moderadamente peligroso	**II**	Modernamente tóxico
III	Ligeramente peligroso	**III**	Ligeramente tóxico
U	Poco probable un peligro agudo	**IV**	Prácticamente no tóxico

Los plaguicidas organofosforados (OP) son un grupo de plaguicidas que se caracterizan por tener en su estructura química una o más cadenas laterales de éster de fosfato (derivados del ácido fosfórico) químicamente reactivo, que consiste en un átomo de fósforo central doblemente unido a un átomo de oxígeno (fosfato) o azufre (tiofosfato), y unido individualmente a dos grupos metoxi (-OCH3) o etoxi (-OCH2CH3). Este grupo de plaguicidas son ampliamente utilizados como agentes de control de plagas en la agricultura, control de vectores de algunas enfermedades y como insecticidas en el hogar (WHO, 2006).

El temefos (O,O,O',O′-tetrametil O,O′-tiodi-p-fenilen bis (fosforotioato)) es un plaguicida perteneciente a los OP, fue desarrollado por *American Cyanamid* entre los años 1963 y 1967. Es comercializado bajo el nombre de Abate® y es utilizado como larvicida para el control de vectores de algunas enfermedades como dengue, zika y chikungunya. Su clasificación toxicológica es controversial; por un lado, la OMS lo clasifica en la clase III: poco peligroso o de baja toxicidad, estableciendo que es inofensivo para los mamíferos, estableciendo una DL_{50} de 4,240 mg/Kg por vía oral en ratas macho y de 4,700 mg/Kg por vía oral en ratones macho. El nivel más bajo de efectos adversos observados (LOAEL) lo estableció en 100 mg/Kg de peso corporal/día hasta por 44 días en ratas; esto se basa únicamente en los signos clínicos causados por el temephos (WHO, 2006). Así pues, la OMS también recomienda el uso del temefos en el agua potable en concentraciones que no excedan 1 mg/L (WHO, 2006). Por otro lado, la EPA clasifica al temefos como plaguicida sistémico en el grupo II: de toxicidad moderada; establece una DL_{50} de 444 mg/Kg vía oral en ratas y una LOAEL de 0.9 mg/Kg/día hasta por 90 días, esto en base a la inhibición de la acetilcolinesterasa (AChE) en ratas de ambos sexos.

La EPA clasifica al temefos como no cancerígeno, además, no se considera tóxico para el desarrollo o la reproducción (EPA, 2008).

A pesar de que el temefos es considerado como un plaguicida de baja toxicidad para los mamíferos, existen diversos estudios que han demostrado lo contrario, incluso utilizando las dosis que son consideradas seguras por algunas organizaciones. Por esta razón, en esta revisión se recopilaron y discuten los principales estudios referentes a la cinética y toxicidad del temefos en mamíferos, mostrando que este plaguicida no es tan inocuo como se cree.

Esta revisión será enviada a la revista *Environmental Pollution* (ISSN: 0269-7491), con un factor de impacto de 8.071 registrado en el año 2020.

2. REVISION BIBLIOGRAFICA

Temephos, a mammal 'safe' organophosphate larvicide: a review of its kinetics and toxicity

Juan Pablo Martínez-Mercado[1], Adolfo Sierra-Santoyo[1], Francisco Alberto Verdín-Betancourt[2], Aurora Elizabeth Rojas-García[2], and Betzabet Quintanilla-Vega[1*]

[1] Departamento de Toxicología, Cinvestav. Ave. IPN #2508, Col. Zacatenco, Mexico City. 07360, Mexico.

[2]Laboratorio de Contaminación y Toxicología Ambiental, Secretaría de Investigación y Posgrado, Universidad Autónoma de Nayarit, 63155, Ciudad de la Cultura s/n, Col. Los Fresnos, C.P. 63190 Tepic, Nayarit, México.

* Corresponding author: B. Quintanilla-Vega. Phone: +52-555747-3310. E-mail: mquintan@cinvestav.mx

Abstract

Temephos (O,O,O',O′-tetramethyl O,O′-thiodi-*p*-phenylene bis (phosphorothionate)) is a larvicide belonging to the family of organophosphate (OP) pesticides, used for the control of different vectors of diseases, such as dengue, zika, chikungunya, and dracunculiasis. The aim of this review was to discuss the available published information about temephos kinetics and toxicity to mammals. Temephos is fast absorbed by the gastrointestinal tract and distributed mainly in the fat tissue. It is extensible metabolized and the reactions that participated in its metabolism are S-oxidation, oxidative desulfuration, and hydrolisis, where the family of cytochrome P450 (CYP) participated. Despite this information, it is still necessary to complete the toxicokinetics of this larvicide. Temephos is mainly eliminated by feces whereas some of its metabolites by urine. The World Health Organization (WHO) classifies it in class III: slightly dangerous with a LOAEL (Lowest observable adverse effect level) of 100 mg/Kg/day for up to 44 days in rats based on the cholinergic symptoms, establishing that it has very low toxicity for mammals. However, different studies have shown that temephos causes toxic effects on mammals. The inhibition of the enzyme acetylcholinesterase (AChE) is one of the main demonstrated effects. This larvicide has also shown genotoxic effects and some adverse effects on male reproduction and fertility have been reported, as well as liver damage, even at safe concentrations. We did an extensive review through several databases for available literature about temephos toxicokinetics, and concluded that there is a need of replanting the use of this larvicide considered safe by international regulators.

Keywords: Temephos, organophosphate pesticides.

Abbreviations: AChE, acetylcholinesterase; AST, aspartate aminotransferase; BPS, bisphenol S; CbxE, carboxylesterase; CYP, cytochrome P450; EPA, Environmental Protection Agency; FMO, flavin monooxygenases; LD_{50}, lethal dose 50; LOAEL, Lowest Observed Adverse Effect Level; OP, organophosphates; SIDP, 4,4'-sulfinyldiphenol; TDF, 4,4'-thiodiphenol; Tem-dox, temephos dioxon; Tem-dox-SO, temephos dioxon sulfoxide; Tem-dox-SO_2, temephos dioxon sulfone; Tem-oxon, temephos oxon; Tem-oxon-SO, temephos oxon sulfoxide; Tem-oxon-SO-OH, temephos oxon sulfoxide mono hydrolyzed; Tem-SO, temephos sulfoxide; Tem-SO_2-OH, temephos sulfone mono hydrolyzed; WHO, World Health Organization.

1. Introduction

Temephos (O,O,O',O′-tetramethyl O, O′-thiodi-*p*-phenylene bis (phosphorothionate)) is a larvicide belonging to the family of organophosphate (OP) pesticides. Its molecular formula is $C_{16}H_{20}O_6P_2S_3$ with a molecular weight of 466.48 g/mol and a melting point of 30-30.5 °C. It decomposes at 120-125 °C and has a solubility of 30 g/L at 25 °C, with a Kow of 80,900 (log Kow = 4.91) (EPA, 2008).

Temephos (Figure 1) is used mainly to kill and control the growth of some vectors, such as mosquitoes, blackfly, and fleas, which transmit diseases, including dengue, zika, chikungunya and dracunculiasis (WHO, 2006). Temephos was synthetized by American Cyanamid between 1963 and 1967, and it is marketed under the name of Abate® as an emulsified compound at different concentrations or in a granulated form, which may contain some impurities, such as iso temephos (1.4%) (WHO, 2008).

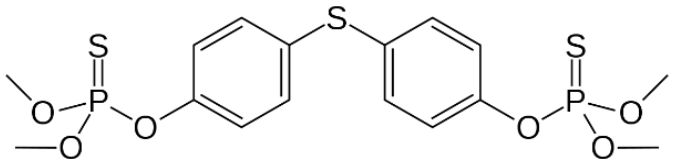

Figure 1. Temephos structure

The toxicological classification of temephos is controversial. The World Health Organization (WHO) classifies temephos in class III: slightly dangerous or of low toxicity, establishing that it is harmless to mammals. WHO has established a lethal dose 50 (LD_{50}) of 4,240 mg/Kg orally in male rats, 4,700 mg/Kg orally in male mice. The LD_{50} for dermal exposure in rats is lower than that observed by oral route, with a value of >4,000 mg/Kg, whereas by inhalation is >4.79 mg/L (WHO, 2006). The dermal LD_{50} in rabbits was estimated to be 2,000 mg/Kg in male and 2,378 mg/Kg in female. The lowest

observed adverse effect level (LOAEL) of temephos reported by the WHO is 100 mg/Kg of body weight/day for up to 44 days in rats, based on the cholinergic clinical signs. Temephos use in drinking water is recommended at concentrations not exceeding 1 mg/L (WHO, 2006; 2008). On the other hand, the Environmental Protection Agency (EPA) classifies temephos as a systemic pesticide in group II: of moderate toxicity. It establishes a LD_{50} of 444 mg/Kg orally in rats and a LOAEL of 0.9 mg/Kg/day for up to 90 days, this based on the inhibition of acetylcholinesterase (AChE) in rats of both sexes. The EPA classifies temephos as non-carcinogenic and it is not considered toxic for development or reproduction (EPA, 2008). This review aimed to present and analyze the available studies found in the literature regarding the kinetics and toxicity of temephos in mammals.

2. Literature search

A comprehensive literature search of eight databases, including: PubMed, American Chemical Society (ASC), Cochrane Library, SciELO, Scopus, Web of Science, EPA, and The Institutional Repository for Information Sharing (IRIS) was performed. Seven key phrases/words were used: temephos, temephos kinetics, abate kinetics, abate larvicide, abate insecticide, toxicology of abate, and toxicology of temephos. The search was conducted until 05/10/2021. All titles and abstracts were read to select those studies performed in mammals, inaccessible and duplicate articles were excluded. Additionally, three unpublished studies performed by our group were included (Figure 2).

In spite of its wide use during five decades, results displayed in the search surprisingly showed the scarce number of studies related to the toxicokinetics of temephos in mammals, including humans. Most papers (n= 651) are related to its use and the

resistance, susceptibility, or toxicity of temephos in some mosquito, such as *Aedes spp*, *Anopheles spp*, and *Culex spp*. Thus, only 24 studies about the kinetics and toxicity of temephos in mammals were considered as the base for this review, including the first ones published in the 1960 decade (Table 1).

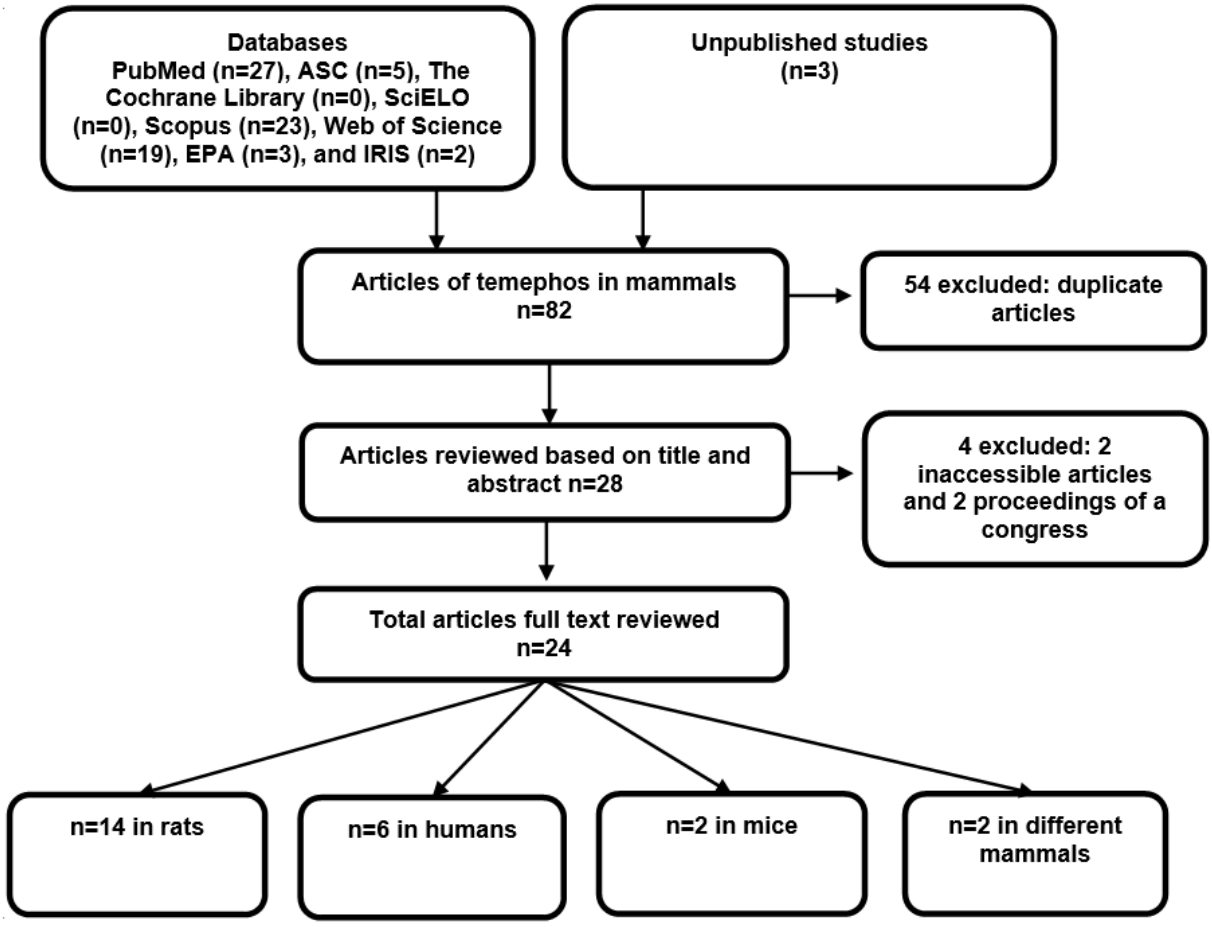

Figure 2. Results of the literature search on the study of temephos in mammals

Table 1. **Summary of temephos studies in mammals.**

Reference	Model	Temephos dose/via	Effect
Aiub et al., 2002	Male Wistar rats	2.14 µM, drinking water	DNA damage in blood cells
Benitez-Trinidad et al., 2015	*In vitro*, human lymphocytes and HepG2 cells	10 µM	Lymphocytes: cytostatic and apoptotic effects and DNA damage HepG2 cells: genotoxic effect (micronuclei)
Blinn, 1969	Male Sprague Dawley rats	970 µg/rat (single dose), orally	Absorption, distribution, biotransformation, and elimination of temephos
Camacho-Hernández, 2019	Male Wistar rats	1 mg/Kg/d for 7 days, orally	AChE inhibition
Ramos-Flores et al. In press	Male Wistar rats	1 and 100 mg/Kg/d for 5 and 7 days, orally	Distribution of temephos in reproductive tissues AChE inhibition Altered sperm quality and fertility rate
Ennin and Franklin, 1979	Male Wistar rats	10 and 300 mg/Kg/d for 4, 7, or 10 days, intraperitoneal	Induction of CYP Alteration of hepatic enzymes
EPA, 2008	Rats	Different doses; LOEL, NOAEL	General information and classification of temephos
Ferguson et al., 1985	Female Sprague-Dawley rats	300 mg/Kg/d for 1 and 5 days, intraperitoneal	Temephos absorption and elimination AChE inhibition
Gaines et al., 1967	Male and female Sherman rats, male guinea pigs, male and female dogs, and male rabbits	1, 10 and 100 mg/Kg/d for 44 and 35 days 50 ppm/dog/day for 129 days	AChE inhibition Hepatic damage
Hernández-Esteris, 2020	Male Wistar rats *In vitro*, rat liver microsomes	50 mg/Kg/d for 3 days, oral 20 µM	Hepatic damage Metabolism
Kim et al., 2020	*In vitro*, mice sperm cells	0.1, 1, 10, and 100 µM	Negative effects on the sperm and male fertility
Kurtz and Weeks, 1979	Male Sprague Dawley rats	1000 mg/Kg/d for 2, 6, 8, 19, and 16 days, intraperitoneal	Decrease in brain AChE and BuChE activity Poor performance of a conditioned response and spontaneous motor activity

Laws et al., 1967	Humans	256 mg/Kg/d for 5 days or 64 mg/Kg/day for 4 weeks	No effect was observed (AChE activity)
Laws et al., 1968	Humans	1 ppm for 19 months, drinking water	No effect was observed (AChE activity)
Martins Laurentino et al., 2019	Female Wistar rats	50 mg/Kg/d during gestational days 6^{th}–13^{th}, gavage	Hyperactivity, stereotyped behavior, and social impairment
Murphy and Cheever, 1972	Male Holtzman rats	2.5 to 250 mg/Kg, orally 5 and 30 ppm in drink water for 7 days	Brain, blood red cells and submaxillary cholinesterase, and liver CbxE inhibition
Rao et al.,1992	Male Wistar rats	8,600 mg/Kg, orally	Brain AChE inhibition
Reyes-Chaparro et al., 2020	Humans	In silico	Biotransformation
Singh et al., 2011	Humans	Occupational exposure (temephos and others OP)	AChE Inhibition DNA damage (lymphocytes) Alteration of hepatic enzymes
Vani et al., 2018	Female Swiss mice	0.0043 and 0.043 mg/Kg/d during all gestation (1^{st} -18^{th}), oral	Distribution Low teratogenic effect Genotoxic effect (micronuclei)
Verdín-Betancourt et al., 2019	*In vitro*, human blood cells	50 µM Tem, 0.1 to 10 µM Tem-SO, 0.05 to 5 µM Tem-dox-SO, and 0.02 to 2 µM Tem-dox-SO_2	AChE inhibition
Verdín-Betancourt et al., 2021	Male Wistar rats	300 mg/Kg (single dose), oral	Absorption, distribution, biotransformation and elimination
WHO, 2006	Male and female rats	10,000 mg/Kg (single dose)	Use and classification
WHO, 2008	Rats, mice and rabbits	Different doses; LOEL, NOAEL	Characteristics and classification

AChE= Acetylcholinesterase; **BuChE**= Butyrylcholinesterase; **CbxE**= Carboxylesterases; **CYP**= Cytochrome P450; **EPA**= Environmental Protection Agency; **LOAEL**= Lowest observed adverse effect level; **NOAEL**= No observed adverse effect level; **WHO**= World Health Organization.

3. Temephos kinetics

3.1. Absorption

The most common types of exposure to this pesticide are occupational and domestic. Temephos is an unstable chemical substance that under environmental conditions is photo oxidized by sunlight, generating some oxidizing metabolites, such as temephos oxon (Tem-oxon) and temephos sulfoxide (Tem-SO) (Lacorte et al. 1996). Kamel et al. (2009) showed that after 72 h of incubation in chlorinated water, temephos is oxidated into stable products, such as Tem-SO, temephos oxon sulfoxide (Tem-oxon-SO), temephos dioxon sulfoxide (Tem-dox-SO), and temephos dioxon sulfone (Tem-dox-SO_2). This simulates the behavior of temephos in domestic water tanks, suggesting that people could be exposed to temephos and some of its oxidized metabolites.

The three main routes of absorption of temephos are inhalation, dermal, and oral. Dermal absorption in rats is approximately 38% (EPA, 2008), while oral absorption has been observed to be rapid, it occurs within the first 5-8 hours, using the compound emulsified in saline solution (Verdín-Betancourt et al., 2021) or in corn (Ferguson et al., 1985) or sesame oil (Blinn, 1969) as a vehicle. Recently, a single dose of temephos (300 mg/Kg, orally) in male rats reached a maximum absorption time (T_{max}) of 2 h (Verdín-Betancourt et al., 2021), a similar value to that found by Ferguson et al. (1985) and Blinn (1969). In addition, an absorption half-life ($T_{1/2\ abs}$) of 0.38 min and a maximum concentration (C_{max}) in blood of 10.4 µg/mL (K_{abs} = 1,812 h-1) were also reported by these authors (Verdín-Betancourt et al., 2021), in agreement to the C_{max} of 10.8 µg/mL reported by Ferguson et al. (1985) using the same dose. The calculated absorption potential for temephos was 4.9, which indicates that the fraction absorbed is almost total

(Verdín-Betancourt et al., 2021); values greater than 1 indicate an almost total absorption (Dressman et al., 1985). However, Blinn (1969) calculated an absorption of approximately 40% of a single dose of temephos (970 µg, orally) administered to rats, this difference could be due to several factors, including a high volume of vehicle used. Lipinski et al. (2001) proposed that physicochemical parameters, such as molecular weight < 500 g/mol, log Kow < 5, and the number of hydrogens bond donors < 5 and acceptors < 10, are good predictors of the absorption potential. Thus, temephos that has a molecular weight of 466.48 g/mol, a log Kow = 4.91 (EPA, 2008), with only three hydrogens to accept and none to donate can be considered to have good absorption.

3.2. Distribution

Verdín-Betancourt et al. (2021) calculated the volume of distribution of temephos as 38.215 mL, which is very large. Temephos is distributed in various tissues, including fat >liver >kidneys >brain. In the study performed by Blinn (1969), a single dose of 970 µg of temephos (Abate) was given via gavage to male Sprague-Dawley rats and at 48 h, temephos was distributed in various tissues: fat >liver >kidneys >stomach >muscle. In this study, the authors also used a single dose of 1357 µg/rat of temephos (Abate) and 72 h after the exposure, the following distribution was observed: fat> intestine> stomach> liver> kidneys> muscle. In both cases, fat was the tissue with the highest temephos concentration. Similar results were observed in the study performed by Ramos-Flores et al. (In press), where temephos was administered at repeated doses of 100 mg/Kg/day for 7 days to male Wistar rats and the larvicide was distributed in the fat >liver >epididymis >testis >brain. On the other hand, Vani et al. (2018) observed that temephos can also be distributed in the placenta in pregnant female Swiss mice after

the administration of temephos at the doses of 0.0043 mg/Kg and 0.043 mg/Kg orally (gavage) during all the gestation (1^{st} -18^{th} day). These studies suggest that temephos has affinity for tissues with high lipid content, possibly due to its physicochemical characteristics.

3.3. Biotransformation

Blinn (1969) reported, for the first time, the presence of some temephos metabolites in urine, feces, and fat of rats treated with single doses of this pesticide (970, 1357, or 3164 µg/rat). These include the S-oxidized metabolite Tem-SO, the deposphorylated products, produced by temephos hydrolysis, and its sulfoxides and sulfones analogues: 4,4'-thiodiphenol (TDF), 4,4'-sulfinyldiphenol (SIDP), and 4,4'-sulfonyldiphenol (SODP), also called bisphenol S (BPS).

Recently, Verdín-Betancourt et al. (2021) identified 11 metabolites of temephos, after exposure to a single dose of 300 mg/Kg of temephos in male Wistar rats. The identified metabolites include oxons, dioxons, monohydrolysates, and the phenolic derivatives: Tem-oxon, Tem-dox-SO_2, temephos dioxon (Tem-dox), Tem-oxon-SO, temephos oxon sulfoxide monohydrolyzed (Tem-oxon-SO-OH), temephos sulfone monohydrolyzed (Tem-SO_2-OH), Tem-dox-SO, SIDP, SODP, TDP, and Tem-SO. Most of them were detected in blood, liver, kidney, brain, and fat tissue 30 min after temephos administration. In blood, only two metabolites were identified, Tem-SO_2-OH (remaining constant at high concentrations after 36 h) and Tem-oxon. On the other hand, in the kidney, Tem-oxon, Tem-dox-SO_2, and Tem-SO_2-OH were detected. In the brain, three metabolites were identified: Tem-SO, Tem-oxon, and Tem-SO_2-OH and in the adipose tissue, Tem-oxon, Tem-SO, and Tem-SO_2-OH were detected. In the liver, the

metabolites Tem-oxon, Tem-SO, Tem-dox-SO2, TDP, and BPS were identified. Thus, Tem-oxon appeared in all samples analyzed. All this information shows that temephos suffers oxidations in the central sulfur (highest proportion) and in the phosphate groups. Temephos as a substrate generated mainly Tem-SO, then Tem-oxon, Tem-oxon-SO, Tem-oxon-SO-OH, TDF, and SIDP. When using Tem-SO as substrate, it generated Tem-oxon-SO (highest proportion), Tem-dox-SO, Tem-dox-SO_2, Tem-oxon-SO-OH, and SODP, which is the final metabolite of phase I biotransformation. Whereas by using Tem-oxon as substrate (very unstable), Tem-oxon-SO, Tem-dox-SO, Tem-dox, and Tem-oxon-SO-OH were generated. On the other hand, the metabolites that were produced by Tem-oxon-SO were Tem-dox-SO (main metabolite), Tem-dox-SO_2, and Tem-oxon-SO-OH. Verdín-Betancourt et al. (2021) made a theoretical proposal of the biotransformation of temephos in rats using phase I enzymes, which include sulfoxide metabolites, sulfones, oxons, and monohydrolyzed compounds (Figure 3). Some of the proposed metabolites have not been identified in the serum, plasma, organs, or tissues of treated animals, but it is believed that they could be intermediaries following the logical sequence of biotransformation reactions.

This biotransformation proposed in rats is partly similar to that proposed by Leesch and Fukuto (1972) in the larva of the *Aedes aegypti* mosquito, where temephos undergoes two consecutives oxidation to produce sulfoxides and sulfones, which subsequently undergo oxidative desulfuration to give rise to oxons, which are then hydrolyzed.

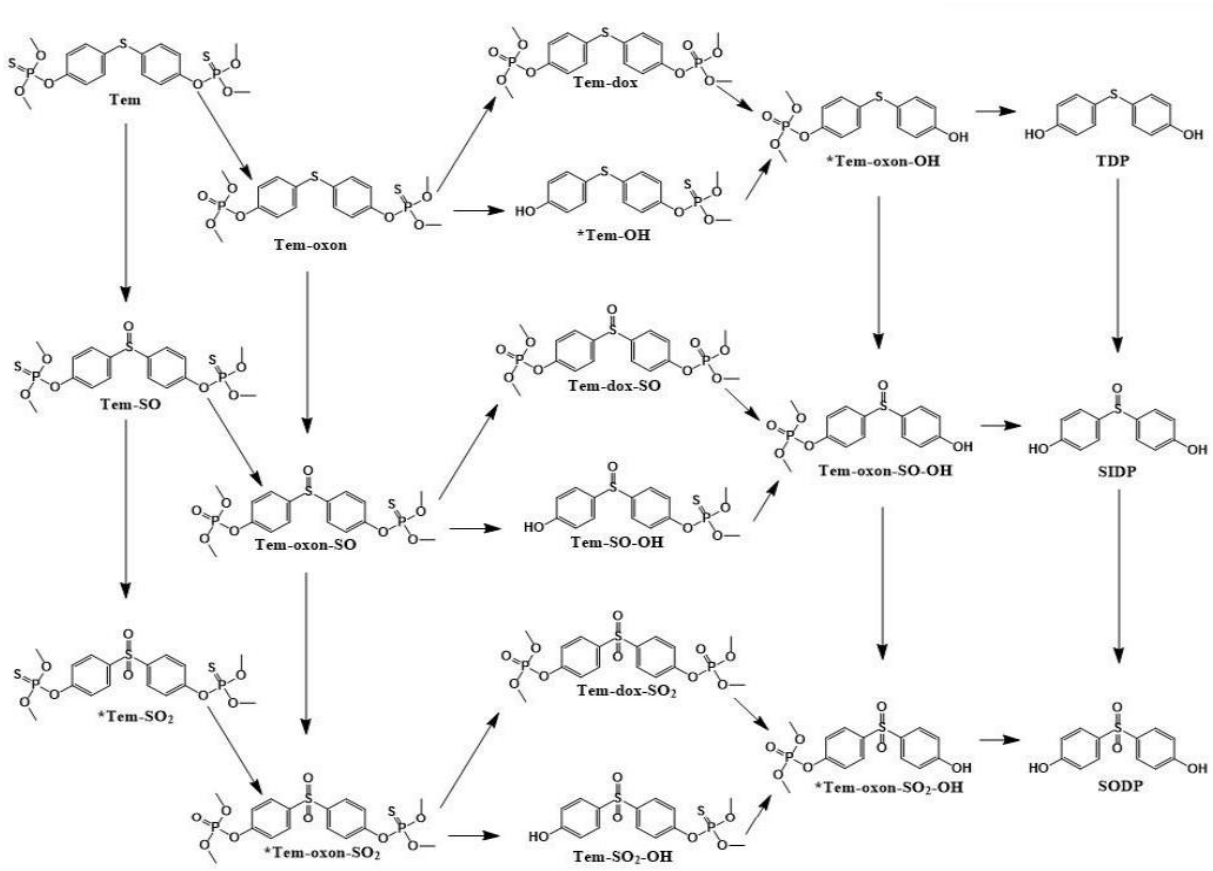

Figure 3. Proposal of a phase I biotransformation pathway of temephos in the rat. *Theoretical metabolites. (Verdín-Betancourt et al., 2021).

There is little information regarding the enzymes that participate in the biotransformation of temephos. Hernández-Esteris (2020) performed a preliminary *in vitro* study in rat liver microsomes. They identified that flavin monooxygenases (FMO) and CYP are the enzymes involved in temephos metabolism. They also identified that CYP3A1/2 and CYP2B1/2 isoforms play an important role in the bioactivation of temephos since they are involved in the formation of mono-oxon and di-oxon derivatives (Hernández-Esteris, 2020). Ferguson et al. (1985) reported that acute and sub-chronic exposures to temephos (Abate) caused inhibition of hepatic mixed-function monooxygenases, but do

not affect CYP expression; whereas, Ennin and Franklin (1979) reported that temephos (Abate) induced hepatic CYP in rats; however, they did not describe which isoforms were affected. Hernández-Esteris (2020) described that temephos differentially affects the regulation of the enzymatic activity of some hepatic CYPs; it discreetly induced the expression of CYP2C11 and 3A1/2, but did not affect CYP1A1, 1A2, 2A1, 2B1/2, or 2E1; however, temephos did not modify its own metabolism *in vitro*.

The only study about temephos metabolism in humans is that performed by Reyes-Chaparro et al. (2020) *in silico*, where the authors reported the possible reactions for both phase I and phase II. The three essential phase I reactions are S-oxidation, oxidative desulfuration, and dephosphorylation, similar to those described in rat metabolism (Verdín-Betancourt et al., 2021). The dephosphorylation is the most likely reaction; it allows the glucuronidation and further excretion of phase II metabolites (Reyes-Chaparro et al. 2020). Whereas Blinn (1969) suggests that the conjugates present in urine of rats treated with temephos are sulfate esters of the hydrolyzed and oxidized products of temephos; these two studies are the only ones about temephos phase II metabolism. Reyes-Chaparro et al. (2020) also suggested the formation of 19 possible intermediate metabolites, among them are oxons, which could cause toxic effects in mammals, and proposed the main CYP isoforms involved in temephos metabolism, including CYP2B6, 2C9, and 2C19, as well as those with lower contribution, such as CYP3A4 and 2D6. These CYPs are not similar as those reported by Hernández-Esteris (2020) in rats.

3.4. Elimination

There is only one study about temephos elimination, reporting the value of blood clearance (Cl) of 3,085.7 mL/h (Verdín-Betancourt et al., 2021). This high value indicates that temephos is rapidly cleared from the blood, which may be due to the great distribution of this compound in different tissues. The fat tissue is particularly the most important; when temephos enters this tissue, it seems to be "kidnapped" and would be hardly released. Temephos extraction ratio was evaluated in other organs, such as the liver, which has the highest clearance capacity with 455.4 mL/h, followed by the kidney with 331.2 mL/h (Verdín-Betancourt et al. 2021).

The $T_{1/2\ elim}$ of this pesticide was one of the first reported toxicokinetic parameters. Blinn (1969) reported a $T_{1/2\ elim}$ of 10 h, Ferguson et al. (1985) a $T_{1/2\ elim}$ of 7 h, and Verdín-Betancourt et al. (2021) a $T_{1/2\ elim}$ of 8.62 h, all these values are very similar. The main routes of elimination of temephos in rats described after an oral administration are feces and urine (Blinn, 1969). The feces being the main route of excretion of the parent compound. It has been described that temephos is eliminated by bile in guinea pigs, based on the 100 times higher amount found in bile compared to blood. This could be due to the physicochemical properties of temephos, including high lipophilicity, neutral, and a high molecular weight (466.48 g/mol). Compounds are excreted in the bile above a threshold molecular weight; in rats, it is approximately 325 ± 50, in guinea pigs is 400 ± 50, and 475 ± 50 in rabbits (Hirom et al., 1972). The urine is the main route of elimination of temephos metabolites, mainly the dihydrolyzed metabolites, such as TDF, SIDP, and BPS (Blinn, 1969); the proportion of them that are eliminated in free and/or conjugated form is not known with certainty.

4. Toxicity

4.1. AChE inhibition

The main toxic effect reported of OP exposure is the inhibition of AChE (Costa, 2008), resulting in the lack of acetylcholine hydrolysis with the accumulation of this neurotransmitter, producing an over-stimulation of muscarinic and nicotinic receptors. The typical symptoms of OP intoxication are agitation, muscle weakness and fasciculation, hypersalivation, and sweating, with respiratory failure, unconsciousness, confusion, convulsions and/or death in severe poisonings (Colović et al., 2013). One of the first studies in which AChE inhibition was reported after temephos exposure was that performed by Gaines et al. (1967) where Sherman rats and rabbits were administered orally with temephos (Abate) at different doses for 44 and 35 days, respectively and reported that at 1 mg/Kg/day, erythrocyte AChE values were maintained normal in both species. In contrast, Ramos-Flores et al. (In press) reported a decrease of 20% on AChE activity on the 3rd day and 29% on the 7th day of temephos administration in male Wistar rats at the same dose for 7 days. The difference may be due to the vehicle used, since Gaines et al. (1967) used an oily vehicle, which suggests that it could influence the absorption capacity due to the high lipophilicity of temephos, compared to saline solution used by Ramos-Flores et al. (In press) that favors a complete absorption (Verdín-Betancourt et al., 2021). Additionally, the difference of animal strain cannot be discarded. The dose of 10 mg/Kg/day of temephos in rabbits caused an inhibition of 26% at 7 days and 47% at the end (35 days) of the study, whereas the rats showed 31% inhibition of AChE after 14 days and 47% after 44 days, however, these animals did not showed symptoms of intoxication (Gaines et al., 1967). On the other hand, rats that

received 100 mg/Kg/day showed 64% inhibition of erythrocyte AChE at 3 days, showing typical symptoms of intoxication, in agreement with results of Ramos-Flores et al. (In press), who reported 70% inhibition at 3, 5, and 7 days using the same dose. However, in this last study, mortalities of 13 and 41% were observed after 5 and 7 days of exposure, respectively. Gaines et al. (1967) also reported 37.5% mortality in rabbits administrated with temephos (Abate) at 100 mg/Kg/day for 5 days. In the same study, male dogs were fed with 50 ppm of temephos and reported an inhibition of erythrocyte AChE of 33% at 7 days and 78% at 129 days; while in females, AChE remained normal until day 60, but 50% was inhibited at 90 days and remained so until 129 days. This sex difference could be because in some species of mammals, females have a faster recovery of erythrocyte AChE than males (Woodard et al., 1994).

In another study performed by Ferguson et al. (1985) in adult female Sprague-Dawley rats exposed to a higher dose (300 mg/Kg) of temephos (Abate) in single and repeated (5 days) exposures showed an erythrocyte AChE inhibition of 67% after 4 h and 47% after 48 h. After the repeated challenge, the maximum inhibition of AChE was 100%, with clinical signs of intoxication, such as muscle fasciculation, salivation, urination, and diarrhea that appeared on day 4^{th} of the treatment and continued through the end of the study. Finally, Kurtz and Weeks (1979) reported a decrease in brain AChE and plasma BuChE activities in rats after exposure to 1000 mg/Kg of temephos (Abate) via intraperitoneal. BuChE is a plasma hydrolase with an unclear physiological function (Chen and Cashman, 2013); its inhibition is associated with some OP exposure, even stronger than AChE, which depends on their affinity (Ventura et al. 2006). Rao et al. (1992) also evaluated brain AChE inhibition and observed a maximum inhibition at 3

days post-exposure to 8,600 mg/Kg of temephos (Abate) orally. A study performed by Murphy and Cheever (1972) using single doses of temephos (Abate) from 2.5 to 250 mg/Kg in male rats showed the potential of temephos to inhibit red blood cells and brain AChE versus liver CbxE, and found that liver CbxE were more sensitive to this larvicide, considering the 50%-inhibition. The reduction of CbxE activity might increase the susceptibility to xenobiotics that are substrate of these enzymes. The same group evaluated this hypothesis in male rats given Abate in drinking water (5 and 30 ppm) for 7 days and then the animals received an intraperitoneal injection of 200 or 400 mg/Kg of malathion (an OP that is a substrate of CbxE and an inhibitor of cholinesterases). The results showed that the brain AChE inhibition was greater in the Abate-pretreated groups compared to the 400 mg/Kg malathion-exposed group, suggesting the potentiation effects of temephos (Abate) pre-treatment.

Studies about AChE inhibition in humans are limited. Singh et al. (2011) showed a decrease in the activity of this enzyme in workers exposed to different OP, including temephos. On the other hand, a study performed in Puerto Rico in volunteers exposed to 256 mg/Kg/d for 5 days or 64 mg/Kg/d for 4 weeks of temephos (Abate), no effect was observed (Laws et al., 1967). A similar result was observed in a group of people exposed to 1 ppm of temephos (Abate) for 19 months, through drinking water, where no inhibition of AChE activity was observed in any of the participants (Laws et al., 1968). These studies are not reliable since, as they reported, people were not always exposed to 1 ppm of temephos because on several occasions the water containers were filled with untreated water (drinking water and rainwater). This lower exposure to temephos is reflected in the decrease of the levels of the Abate-derived compound detected in urine.

Finally, an *in vitro* study conducted to evaluate the inhibition of AChE in human blood cells incubated with several oxidized temephos products concluded that, the highest the degree of temephos oxidation, the greatest inhibition of AChE. Thus, Tem-dox-SO_2, which is the most oxidized metabolite, had the highest potential of inhibition, even greater than that of paraoxon, one of the most neurotoxic OP. This metabolite also inhibited serum BuChE with values similar to paraoxon (Verdín-Betancourt et al., 2019).

4.2. Behavioral effects

Some studies have reported behavioral effects of temephos. Martins Laurentino et al. (2019) reported that temephos caused hyperactivity, stereotyped behavior, and social impairment in rats after prenatal exposure to temephos 50 mg/Kg/day (diluted in distilled water) between gestational days 6^{th} -13^{th} by gavage. The authors concluded that this behavior is similar to that shown in some models of neurobiological diseases, such as attention deficit hyperactivity disorder (Zhou et al., 2017) and autism spectrum disorders (Laugeray et al., 2014). On the other hand, Kurtz and Weeks (1979) described that rats exposed to temephos (Abate) 1000 mg/Kg (intraperitoneal injection) showed a poor performance of a previously conditioned avoidance response, at 6 days after exposure, but not at 2, 8, 10, or 16 days. They also observed that the deterioration of avoidance in animals that received this dose of temephos was accompanied by the inhibition of BuChE, and erythrocyte and brain AChE, as well as a decrease in spontaneous motor activity.

4.3. Genotoxicity

Based on the few studies performed in rats and rabbits, the WHO considered temephos as a non-genotoxic nor mutagenic agent, however, some of the main studies in this

evaluation did not comply with the good laboratory practices (WHO, 2006). A study carried out in male Wistar rats exposed to 2.14 µM of temephos in drinking water (a concentration similar to that used to combat the mosquito) reported that temephos produced DNA damage in blood cells (Aiub et al., 2002). On the other hand, Vani et al. (2018) reported that temephos produced micronuclei in blood cells of pregnant female mice after 48 h exposure to 0.043 mg/Kg/day throughout the gestation period. Singh et al. (2011) observed DNA damage in lymphocytes of workers exposed to pesticide mixtures (pirimiphos methyl, chlorpyrifos, temephos, and malathion) using the comet assay, these results were also observed in sprayers occupationally exposed to OP (temephos, chlorpyrifos, malathion, parathion, methamidophos, and acephate) and pyrethroids (Zepeda-Arce et al., 2017). In addition, Benitez-Trinidad et al. (2015) reported, through an *in vitro* study in human lymphocytes and HepG2 cells (cells with metabolic capacity) incubated with temephos (10 µM), that temephos has cytostatic and apoptotic effects, as well as the ability to produce DNA damage (evaluated by the comet assay) in lymphocytes. Whereas, a genotoxic effect, evaluated by the presence of micronuclei, was observed only in HepG2 cells, suggesting that temephos metabolism is involved in this effect.

4.4. Reproductive effects

There is limited information about reproductive effects of temephos. Recently, Camacho-Hernández (2019) reported that exposure to 1 mg/Kg/day/7 days of temephos did not produce adverse effects on sperm quality parameters. However, rats exposed to 100 mg/Kg/day/7days caused a significant decrease in sperm motility (30%) and viability (7.5%); similar effects were observed in sperm motility (12%) and viability

(5%) after 5 days of exposure (Ramos-Flores et al., In press). In addition, temephos altered the structure of sperm chromatin at 7 days of exposure and produced a 12% increase in lipoperoxidation in rats exposed to 100 mg/Kg/day/7 days, effects not observed at 5 days of exposure. Any of the treatments altered the sperm morphology or caused DNA damage. These results suggest that the time of exposure is important in the damage caused by temephos, due to the kinetics of this larvicide. Interestingly, a decrease of 30% in the fertilization potential of spermatozoa from rats exposed to temephos at 100 mg/Kg/day/5 days was observed, as well as fragmented zygotes fertilized with spermatozoa from temephos-treated rats (Ramos-Flores et al., In press), a dose that is considered the LOAEL by the WHO.

One probable mechanism of action of temephos to alter the fertilization potential is by altering the acrosome reaction, since this parameter is considered a good predictor of the fertilization potential (Tello-Mora et al., 2018). The acrosome reaction has been shown to be a target of other highly toxic OP reducing the male fertile capacity (Piña-Guzmán et al., 2006; Urióstegui-Acosta et al., 2014). Also, a recent *in vitro* study performed in mouse spermatozoa incubated with different concentrations of temephos (0.1-100 µM) showed a significant decrease in motility, sperm capacitation, Tyr phosphorylation, activity of protein kinase A, and intracellular adenosine triphosphate levels, whereas the spontaneous acrosomal reaction increased. The authors also reported decreased fertilization rate and altered early embryonic development (Kim et al., 2020). This study supports our hypothesis that temephos damages sperm cells by altering the acrosome *in vivo*. It has been reported that the two most common mechanisms of action of OP in sperm cells are phosphorylation (Piña-Guzmán et al.,

2005) and oxidation (Uriósteguí-Acosta et al., 2020) of proteins; therefore, the molecular mechanism of action of temephos on the fertilization rate remains to be elucidated. On the other hand, Vani et al. (2018) showed that at an exposure of 0.0043 mg/Kg of temephos in pregnant female Swiss mice, the size of the fetuses increased without external malformations, whereas changes were induced in the sternum at a dose of 0.043 mg/Kg. All this information suggests that temephos can produce alterations in male sperm function and embryo development at low “safe” concentrations.

4.5. Hepatic effects

Some studies have revealed the hepatic toxicity of temephos. Gaines et al. (1967) administered rabbits to temephos (Abate) at the dose of 100 mg/Kg/day for 5 d orally and reported diffuse and focal necrosis of the liver in treated animals. WHO (2006) reported that a single dose of temephos (10,000 mg/Kg) administered to male and female rats caused a discolored liver of all treated male animals accompanied of bleeding in the intestinal lumen, whereas, no adverse findings were seen in females. Ennin and Franklin (1979) reported a slight decrease in glutamate dehydrogenase levels and no changes in liver aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase, or lactate dehydrogenase activities after intraperitoneal administration of temephos (Abate) at doses between 10 and 300 mg/Kg for 4, 7, or 10 days in rats. Whereas, Singh et al. (2011) reported an increase in liver AST, ALT, and alkaline phosphatase activities in workers exposed to OP, including temephos. Results from our group showed a decreased relative liver weight in animals exposed to a dose of temephos equivalent to the LOAEL (100 mg/Kg/day/5 days; gavage), suggesting a hepatotoxic effect (Ramos-Flores et al., In press). Temephos (Abate) inhibits liver CbxE

(Murphy and Cheever, 1972), which could generate alterations in the homeostasis of hepatic lipids (Lian et al., 2018; Wang et al., 2018). In addition, preliminary results from our group in adult male Wistar rats exposed to 100 mg/Kg/day/7 days showed a 12% decrease in the relative weight of the liver, an increase in direct, indirect, and total bilirubin levels, a lipid imbalance characterized by a decrease in triglycerides, very low-density cholesterol, and total lipids (Hernández-Esteris et al., 2020). A recent study performed in rats exposed to temephos at a dose of 50 mg/Kg/d for 3 days reported a 12% increase in the liver weight, although neither the microsomal protein content nor the total hepatic CYP concentration were altered (Hernández-Esteris, 2020). In addition, in an *in vitro* study where liver microsomes from rats previously treated with temephos (20 µM) were used, the biotransformation of temephos towards its oxidized metabolites was reduced with respect to untreated microsomes, suggesting that temephos exposure affects the enzymes responsible for its own metabolism (Hernández-Esteris, 2020).

5. Conclusions

Temephos is a larvicide belonging to the OP family, used mainly for the eradication of some vectors that cause illnesses, such as *Aedes aegypti*. The WHO considers this pesticide of low toxicity for mammals. The complete metabolism of this pesticide is still unknown, however, its metabolism by phase I enzymes has already been demonstrated in rats, which include FMO and CYP (CYP3A1/2 and CYP2B1/2). Temephos undergoes different oxidations to form its analogues oxones, sulfones, and sulfoxides, as well as hydrolysis to eliminate its phosphate groups. The metabolism of temephos by phase II enzymes has not yet fully described. The inhibition of AChE is one of the main toxic

effects reported by this larvicide at the LOAEL, a “safe” dose. Although the WHO considers this compound not-genotoxic, some studies have showed DNA damage even at concentrations used to combat the mosquito. Liver damage is another toxic effect produced by this pesticide, which induces changes in the weight of this organ and alterations in liver enzymes. There are no studies in humans about temephos reproductive effects; however, some animal studies have reported male adverse effects, including decreased fertilization rates at temephos concentrations considered safe. Although more studies are needed to fully know the toxicity of this pesticide in mammals, literature already published shows that temephos causes toxic effects, even at the LOAEL. Therefore, this dose should be reconsidered, as well as new strategies to reduce or replace the use of this larvicide.

Author contributions

Each author contribute on the following:

Martínez-Mercado JP: did the literature search, wrote the first draft and participated in the final version of the review

Sierra-Santoyo A: Writing - Review & Editing

Verdin-Betancourt FA: Writing - Review & Editing

Rojas-García AE: Writing - Review & Editing

Quintanilla-Vega B: conceptualized the idea, organized and supervised the writing of the review, and ensures that the descriptions are accurate and agreed by all authors

Conflicts of interest

The authors declare that there are no conflicts of interest to declare.

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3. DISCUSIÓN Y CONCLUSIONES

Después de una búsqueda en las bases de datos más conocidas, un total de 24 artículos relacionados con la cinética y la toxicidad del temefos fueron elegidos y revisados para su integración en esta revisión. Esta búsqueda mostró que la gran mayoría de los estudios relacionados con este larvicida se enfocan en el uso, resistencia, susceptibilidad y toxicidad producida en algunos mosquitos. Lo anterior demuestra que se requieren hacer más estudios de este larvicida en mamíferos, que, aunque no son la especie blanco, se utilizan como modelos para estudiar los efectos en los seres humanos.

Son pocos los estudios que se tienen sobre la cinética del temefos. Se sabe que tiene una absorción rápida por vía oral (Blinn, 1969; Ferguson et al., 1985; Verdín-Betancourt et al., 2021) y un gran volumen de distribución; distribuyéndose en varios órganos y tejidos como la grasa, hígado, cerebro, testículo, epidídimo y placenta (Blinn, 1969; Ramos-Flores et al., En prensa; Vani et al., 2018), presentando mayor afinidad por tejidos con alto contenido lipídico. Se han logrado identificar 11 metabolitos del temefos, los cuales incluyen oxones, dioxones, compuestos monohidrolizados y derivados fenólicos (Verdín-Betancourt et al., 2021), todos estos producidos por la biotransformación del temefos mediante reacciones de S-oxidación, desulfuración oxidativa y/o desfosforilación (Reyes-Chaparro et al., 2020; Verdín-Betancourt et al., 2021). Aún no se han descrito las enzimas que participan en estas reacciones, aunque se sugiere que algunas monooxigenasas de flavina (FMO) y citocromos P450 (CYP) son las que participan en estas reacciones de fase I (Hernández-Esteris, 2020; Reyes-Chaparro et al., 2020). Por su parte, el metabolismo de fase II aún no ha sido descrito.

Se sugiere que después de la desfosforilación del temefos, este sufre una glucuronidación y es excretado (Reyes-Chaparro et al., 2020); sin embargo, también se sugiere que los conjugados pueden ser ésteres de sulfato de los productos hidrolizados y oxidados del temefos (Blinn, 1969). Aún es necesario realizar más estudios para poder completar el metabolismo de este plaguicida, tanto de fase I como de fase II.

Algunos estudios mostraron que al igual que otros OP, el temefos también tiene la capacidad de inhibir la enzima AChE, disminuyendo su actividad (Ferguson et al., 1985; Kurtz and Weeks, 1979; Rao et al.,1992; Singh et al., 2011), incluso a concentraciones similares a la LOAEL propuesta por la EPA (Camacho-Hernández, 2019) y por la OMS (Ramos-Flores et al., En prensa), esto en tiempos cortos de exposición. También se demostró que los metabolitos más oxidados son los que tienen mayor capacidad de inhibir esta enzima (Verdín-Betancourt et al., 2019).

Por otro lado, los estudios de genotoxicidad mostraron que el temefos tiene la capacidad de producir daño en el ADN (Benitez-Trinidad et al., 2015), tanto en concentraciones empleadas para combatir al mosquito (Aiub et al., 2002), como de forma ocupacional (Singh et al., 2011; Zepeda-Arce et al., 2017). Igualmente, induce la formación de micronúcleos a concentraciones menores a las LOAEL propuestas por la EPA y la OMS (Vani et al., 2018).

Aunque la información sobre la toxicidad del temefos en la reproducción masculina es muy limitada, se sabe que este larvicida afecta la calidad espermática y el potencial de fertilización de los espermatozoides a concentraciones consideradas seguras por la OMS (Ramos-Flores et al., En prensa). También se demostró que tiene la capacidad de alterar algunos parámetros en el espermatozoide de importancia para la fertilización, como son la capacitación espermática y la reacción acrosomal (Kim et al., 2020). Esto

sugiere que el temefos produce alteraciones en el espermatozoide que afectan la reproducción, pero aún no se sabe cual es el mecanismo por el cual produce estos efectos.

El hígado es otro de los órganos que se ve afectado por el temefos, produciendo necrosis (Gaines et al., 1967) y una disminución en su peso (Ramos-Flores et al., En prensa) a la dosis de la LOAEL propuesta por la OMS. También se observó que este larvicida produce alteraciones en algunas enzimas hepáticas, tanto de importancia fisiológica (Ennin and Franklin, 1979; Singh et al., 2011), como enzimas que participan en la biotransformación de xenobióticos (Murphy and Cheever, 1972; Hernández-Esteris, 2020). Se observó también que produce una alteración en la homeostasis de los lípidos (Hernández-Esteris et al., 2020). Todos estos datos sugieren que el hígado es un órgano blanco del temefos que amerita mayor investigación.

A pesar de que son pocos los estudios referentes a la cinética y toxicidad del temefos, estos muestran su toxicidad sobre los mamíferos, incluso a las concentraciones consideradas como seguras por diferentes agencias, lo que sugiere reevaluar la toxicidad del temefos para los seres humanos.