Review

Exposure to organophosphate pesticides and thyroid function: A systematic review

Exposition aux pesticides organophosphorés et fonction thyroïdienne : Une revue systématique

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ABSTRACT

Organophosphate pesticides (OPs) are widely used for various agricultural, industrial and domestic purposes. Long-term exposure to these products leads to several health problems, including a potential disruption of the thyroid gland as suggested by recent studies. The purpose of this review is to examine the published scientific evidence on this effect. A systematic review of articles published between 1970 and 2019 was conducted using the PubMed and ScienceDirect databases. Twenty-two studies were included in this review: 4 human studies, 16 *in vivo* studies and 2 *in vitro* studies. Almost all *in vivo* studies have shown impaired thyroid function with decreased T3 and T4 hormones and increased TSH levels, reflecting hypothyroidism. The findings from human studies converge with *in vivo* tests regarding the presence of a disruption in thyroid hormones, but diverge in the direction of this disruption. In the 2 *in vitro* studies, dysthyroidism was induced by OPs, although the diagnostic tools were different, in terms of cell lines or parameters explored. Studies on the impact of OPs on thyroid function remain insufficient. More clinical and empirical studies are needed with a standardized methodology that allows the comparison of the results of several studies.

KEYWORDS: Organophosphate pesticides, Endocrine disruption, Thyroid hormone

RESUME

Les pesticides organophosphorés (OP) sont largement utilisés à diverses fins agricoles, industrielles et domestiques. L'exposition à long terme à ces produits entraîne plusieurs problèmes de santé, notamment une perturbation potentielle de la glande thyroïde, comme le suggèrent des études récentes. L'objectif de cette revue est d'examiner les preuves scientifiques publiées sur cet effet. Une revue systématique des articles publiés entre 1970 et 2019 a été réalisée en utilisant les bases de données PubMed et ScienceDirect. Vingt-deux études ont été incluses dans cette revue : 4 études évaluant l'effet chez l'homme, 16 études *in vivo* et 2 études *in vitro*. La quasi-totalité des études *in vivo* ont montré une altération de la fonction thyroïdienne avec diminution des hormones T3 et T4 et augmentation de la TSH, témoignant d'un hypothyroïdisme. Les conclusions des études chez l'Homme convergent avec les essais *in vivo* quant à la présence d'une perturbation au niveau des hormones thyroïdiennes, mais sont divergentes quant au sens de cette perturbation. Dans les 2 études *in vitro*, une dysthyroïdie a été induite par les OP, bien que les outils de diagnostic aient été différents, en termes de lignées cellulaires ou de paramètres explorés. Les travaux étudiant l'impact des OP sur la fonction thyroïdienne restent insuffisants. Des études cliniques et empiriques supplémentaires sont nécessaires avec une méthodologie standardisée permettant la comparaison des résultats.

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Introduction

The thyroid gland plays a crucial role through the secretion of hormones. Thyroid hormones are vital for a multitude of physiological functions, including metabolic effects, potentiating adrenaline and growth hormone and are essential for the ossification and maturation of organs. In the central nervous system, they lead to maturation and connections between neurons. In the autonomic nervous system, they potentiate the effects of catecholamines, accelerate transit and possibly have a trophic action on the skin and appendages [1].

The thyroid is probably the most vulnerable endocrine gland to changes in its environment. Chemical compounds that have a negative impact on thyroid homeostasis are called thyroid disruptors. These fall within the broader framework of endocrine disruptors, a concept that has become an emerging concern in public health. Thyroid disruptors can be of natural or synthetic origin. Chemical compounds of natural origin have always existed in the environment and have goitrogenic properties known for a long time. Exposure is generally dietary: thiocyanates and isothiocyanates present in cruciferous vegetables (cabbage, broccoli, turnip, radish, etc.) and in tobacco, cyanogenic glucosides present in some roots or tubers (cassava, sweet potato), disulfides (onion, garlic), isoflavones (millet, sorghum, beans, soy). Environmental synthetic thyroid disruptors are at the center of this problem. These are products from the phyto-agro-food industry or industrial products, released in large quantities into the environment. The list of these products is long and is growing every year. All areas are affected by food industry, cosmetics, pesticides and persistent organic pollutants, industrial products, paints, solvents, plastics (phthalates, bisphenol A), heavy metals, flame-retardants, dioxins, etc. [2,3].

Pesticides, potential endocrine disruptors, have been increasingly used in recent years to overcome the food needs of the population. Among the pesticide classes, organophosphates are an important issue for health since they are widely used because of their cost-effectiveness, their wide and attractive spectrum of action and their low persistence. These products are used mainly for the protection of plants in agriculture and horticulture as well as in public hygiene in the campaigns of disinsectization and locust control, in the forest field, landscape maintenance, in veterinary practice, etc. Today, many OPs are marketed worldwide in the form of thousands of different products [4].

To date, the most frequently reported health effects related to chronic exposure to OPs are cancers, reproductive disorders and neurological disorders [5]. However, other effects have been mentioned in the literature, notably with the recent emergence of the concept of "endocrine disruption." Many publications report that exposure to OPs changes thyroid signaling to relevant levels in living beings. In view of the equivocity of the results emerging from the literature, the present work aims to clarify the current state of knowledge about the impact of OPs on thyroid function.

Methods

An in-depth review of Anglo-Saxon scientific research was carried out independently by five reviewers, through a systematic review of all the original articles that investigated the thyroid disruptive effect of OPs, for the period from January 1970 until December 31, 2019.

Literature searches were performed in PubMed and ScienceDirect databases, using the following terms (MeSH terms): *pesticides organophosphates, non-persistent insecticides, thyroid stimulating hormone, t4 thyroid hormone, t3 thyroid hormone, and thyroid gland.* Retrieved studies were examined to determine whether they met or did not meet our inclusion criteria before checking the articles.

Inclusion criteria as criteria that guided the selection process of the articles, we included all the epidemiological or experimental studies that examined the association between exposure to OPs and thyroid function, written in English, with access to the full text.

Exclusion criteria: all experimental studies on histopathological changes in the thyroid following exposure to OPs, without exploration of thyroid function, were excluded. This will be the same for all publications that study the effects of exposure to different classes of pesticides, including OPs, on thyroid function without specifying the part of the latter.

It should be noted that the article selection process is based on the methodological recommendations provided by the Preferred Reporting Items for Systematic reviews and Meta-Analyzes (PRISMA) grid, drawing up the different guidelines for conducting meta-analyzes and systematic reviews of the literature.

Results

PubMed produced 29 results from the search criteria, while ScienceDirect produced 26 articles, for a total of 55 articles. After deletion of 4 duplicatives, 22 articles were rejected if it was determined from the title or the abstract that the study failed to meet the inclusion criteria as indicated in the flow diagram **(figure 1)**. After this screening, 7 articles were also excluded because they did not meet finally the initial criteria "false-positive" (n=6) or were not accessible (n=1). Finally, a total of 22 studies were eligible for inclusion in this systematic review: 4 studies evaluated the effect on humans, 16 *in vivo* studies and 2 *in vitro* studies. The results of the research are expressed in the form of tables **(tables 1-3)** including the identification of the study, the methodology, the evaluation parameters as well as the results and the conclusion of each study.
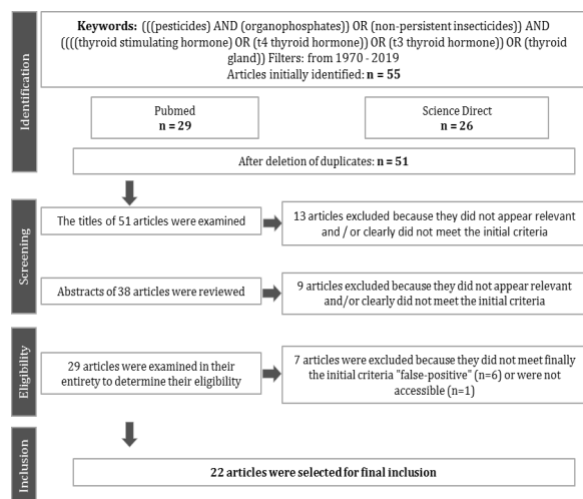

Figure 1. Literature selection diagram for the systematic review.

Discussion

In vivo studies

Sixteen *in vivo* studies were eligible for inclusion in this review. All these studies are randomized and discuss changes in thyroid function induced by exposure to OPs.

With regard to test protocols, excepting the studies by Maiti et al. **[9]** and Rawlings et al. **[11]** conducted on chicks and sheep respectively, the other experiments mainly focused on fish and rodents for several reasons. For fish, the main non-target species that has economic importance, the corollary problem of the effect of water pollution on fish has lead researchers to elucidate the impacts of various pesticides on different fish species.

Concerning the order of rodents, which is the cornerstone of research devoted to human pathologies, the studies included in our review used rats and mice, in mature or just postnatal age, males and females. The latter are used in particular for the study of the *in utero* effect.

Several OPs were used in the trials: malathion, methamidophos, monocrotophos, chlorpyriphos, dimethoate, fenitrothion, phosphamidon. Their choice, among other OPs, in the study of the disruptive effect of thyroid function seems to be related to their wide commercialization and use.

Some studies have focused exclusively on OPs (a single molecule or two associated molecules) while others on mixtures of OPs with other types of pesticides such as lindane, pentachlorophenol, carbofuran, triallate, trifluralin, 2,4-D.

The route of administration of the pesticides studied differs from study to another; oral, intraperitoneal and subcutaneous routes have been described. It seems that the choice of the route of administration depends essentially on the product studied. The example of Chlorpyriphos which is administered orally because it is moderately toxic by this route, and severely toxic by the dermal route in rats **[28]**.

An adjuvant has been used in some studies for the administration of the pesticide: peanut oil for Chlorpyriphos *per os* and Dimethylsulfoxide (DMSO) for the same pesticide by subcutaneous route. Peanut oil is a vegetable oil used for its neutral taste and its ability to increase the absorption of xenobiotics, while DMSO is an organic solvent used to promote the

transport of xenobiotic in cells and also for its anesthetic and antibacterial properties.

The animals were exposed to pesticides either acutely or chronically. The first situation undoubtedly leads us to review conventional toxicology approaches such as "the dose makes the poison". On these bases, it would be difficult to predict the effects of low doses from the effects observed at high doses. One might even expect that at low doses the effects will be more pronounced than at high doses by giving non-monotonic dose-response curves (NMDRC), a model known for several other endocrine disruptors as well as for hormones [29].

The administration of pesticides was carried out at doses calculated according to the weight of the animals, over a period of a few days or even a month to reproduce chronic exposure. However, in the study conducted by Satar et al. [15], endocrine disruption was assessed after acute exposure to a lethal dose of metamidophos to rats, followed by antidotal treatment with pralidoxime and atropine.

A control group was used in all studies (thus referred to as exposed/not exposed), which gives more credibility to the results obtained.

Regarding the parameters for assessing thyroid disruption, thyroid hormones (T3, T4) and TSH, the best markers for monitoring thyroid function, were measured in almost all studies (15/16). Some studies take, in addition, as an identifying factor for thyroid dysfunction, the activity of iodothyronine deiodinases type 1 (D1), 2 (D2) and 3 (D3), the mRNA expression of indices involved in the HPT axis, the thyrotropin releasing hormone (TRH), etc. Otherwise, in the Saxena and Mani study [6], only the level of protein-bound iodine was explored.

Concerning the results of the studies, only one finding stands out, that of the alteration of thyroid function with, in almost all studies, a decrease in T3 and T4 hormones and an increase in TSH, indicating hypothyroidism. This reinforces the hypothesis that OPs are likely to have, in the short and long term, an endocrine disrupting effect, among others, on the thyroid gland.

However, even if animal trials are much less time-consuming and their results may be relevant to humans, they should be supported by other trials and epidemiological studies in different categories of populations, taking into account all aspects of exposure to these toxic substances.

Studies in humans

The literature search for studies carried out on populations exposed to OPs, in search of a consequent thyroid disruption, is not very fruitful. Only four studies were selected for this work since they specifically discuss the issue. In fact, scientific knowledge on this subject is constantly increasing in recent years and will never be sufficient given the specificity of one or the other study as regards the population studied, the context of exposure, the molecule involved, the explored gland, etc. Indeed, many studies have not been included in our review since they study endocrine disrupting chemicals except OPs, and the disruption of other glands in the body and hormonal profiles except the thyroid gland.

The studies selected were carried out in occupationally exposed populations, namely floriculture workers in the Lacasaña et al. study [23], as part of an etiological diagnosis of sub-infertility or sterility as in the Meeker et al. study [22], in pregnant women in the work by Wang et al. [25] or in the general population in the study by Fortenberry et al. [24]. That said, the problem of endocrine disruptors is ubiquitous; exposure to these products occurs at home, in the office, on the farm, in the air we breathe, in the water and food we eat.

Meeker et al. and Lacasaña et al. studies [22,23] concerned only the male sex (men investigated for infertility or sterility, workers men occupationally exposed). Wang et al. [25] studied the issue in pregnant women.

These are longitudinal studies that assess the problem over a long period of time. The duration of studies is of the order of several years (3 to 4 years). In addition, Lacasaña et al. study [23] compared the exposure of the same workers between two agricultural periods, the rainy season when pesticides are used heavily, and the dry season, a period of low pesticide application.

The samples are considered representative given their size: 3249 subjects in the Fortenberry et al. study [24], 322 men in the Meeker et al. study [22], 136 in the Lacasaña et al. study [23] and 325 pregnant women in the Wang et al. study [25].

To study the pesticide-endocrine disruption relationship, the authors used measurement tools to assess the intensity of exposure to OPs and to measure the degree of thyroid disruption. For the exposure assessment, Meeker et al. [22] and Fortenberry et al. [24] used 3,5,6-trichloro-2-pyridinol (TCPy),

metabolite of chlorpyriphos and chlorpyriphos-methyl, while Lacasaña et al. **[23]** and Wang et al. **[25]** measured dialkylphosphates (DAP), common metabolites of OPs. In addition to TCPy, Meeker et al. **[22]** measured the carbaryl metabolite 1-naphthol (1N) and, in addition to DAP, Lacasaña et al. **[23]** measured the serum enzymatic activity of paraoxonase-1 (PON1) and p, p'- dichlorodiphenyldichloroethylene (p, p'-DDE), the main metabolite of DDT. These parameters are potential confounders for the assessment of thyroid disruption. For the evaluation of the disruption of the thyroid function, the authors used the assay of thyroid hormones (T3, T4, TSH).

The conclusions of these studies are quite disparate. Meeker et al. **[22]** describe an increase in TSH levels with a decrease in T4 levels linked to an increase in the levels of the metabolite TCPy. Environmental exposure to chlorpyriphos, chlorpyriphos-methyl could thus be associated with hypothyroidism in men. Lacasaña et al. **[23]** report an association between an increase in total dimethylphosphate (ΣDMP) levels in urine and an increase in TSH and T4 hormones in serum on the one hand, and a decrease in serum T3 levels on the other.

Wang et al. **[25]** describe a positive association of urinary DAP levels with free T4 levels and a negative association with TSH levels. The results of Fortenberry et al. **[24]** goes in the same direction since exposure to OPs (chlorpyriphos and chlorpyriphos-methyl) was responsible for a significant increase in T4 levels and a decrease in TSH levels.

These data imply that the epidemiological literature emphasizing dysthyroidism associated with exposure to OPs is still poor, despite the evidence provided by animal studies. Contrary to the latter, the conclusions of studies in humans are divergent as to the direction of the disruption but converge with the *in vivo* tests on the presence of a disruption in thyroid hormones.

In vitro studies

Similar to studies in humans, there are few studies on the relationship between exposure to OPs and a consequent thyroid disruption *in vitro*. Only two studies seem to answer the question.

Both studies were not specifically conducted for OPs; Ghisari et al. study **[26]** analyzed the action of thirteen pesticides of different classes, including malathion, and that of Porreca et al. **[27]** investigated the effect of ethylene thiourea in parallel with that of chlorpyriphos.

Cultures were carried out on different cell lines. GH3 cells from rat somatotropic pituitary tumor were used in the Ghisari et al. **[26]** experiment and PCCl3 cells, which are immortalized thyrocytes from rats, in Porreca et al. work **[27]**.

The evaluation parameters for the two studies are distinct: GH3 cell proliferation assay and biological test of the AhR-sensitive luciferase reporter gene for the study of Ghisari et al. **[26]**, and analysis flow of high RNA sequencing data (RNAseq) and analysis of ingenuity pathways (IPA) for the study of Porreca et al. **[27]**.

Although the diagnostic tools were different for both studies, either in cell lines or explored parameters, thyroid dysfunction was induced by the OPs in the two cases. Thus, *in vitro* studies are a powerful tool for predicting adverse effects *in vivo* although they do not fully summarize the biocomplexity of an animal. In addition, *in vitro* prediction of unexpected results could allow better planning of *in vivo* experiments.

Conclusion

The first observation to make is the bibliographic void concerning the study of the association between exposure to OPs and disruption of the thyroid function, without minimizing the interest in all endocrine disruptors and their effects on health. Whether in animals or humans, studies remain insufficient since they are limited to a route of administration, a molecule or a mixture of products containing OPs as well as other classes of pesticides (co- exposures), a very precise population and therefore a well-defined exposure context, an exposure during a very precise period of life (*in utero* looking for a problem of fetal development or adulthood…) etc. The literature will therefore remain eager for any other study to expand the database on this subject and in particular, to explain the mechanisms of this effect which remain poorly understood since the dose-effect relationship seems non-monotonous.

If animal tests were unanimous that OPs would cause hypothyroidism, the conclusions of studies in humans have remained less pronounced and disparate although they all fall back on the existence of a disruptive effect on the thyroid. More clinical and empirical studies are then needed to support more specific conclusions, explain the mechanisms of action and explore the clinical significance of these alterations in thyroid function. In addition, a standardized methodology is necessary to allow the comparison of the results of several studies.

Table 1. Summary table of *in vivo* studies analyzing the OP-thyroid disruption relationship.

Study	Methodology	Assessment parameters	Results	Conclusion
[6]	-Collect of 100 adult specimens of *C. punctatus* -Exposition of 30 fish to subtoxic concentrations of fenitrothion (1.5 ppm) or carbofuran (5 ppm) -At least, six specimens of fish remaining with the two pesticides, each belonging to experimental and control groups, were collected at 30-day intervals for 120 days.	Protein-bound iodine (PBI)	- At D30, PBI levels in treated and control fish did not vary significantly -At D60, PBI levels were lower in fish treated with fenitrothion -At D90, PBI levels of all the treated fish were lower	Exposure to fenitrothion leads to a decrease in plasma PBI levels due to atrophy of the thyroid follicles which limits the synthesis and/or release of thyroid hormones
[7]	-Collect of females of *H. fossilis* during prespawning phase -Division of the fish into 5 lots of 5 fish each. 4 lots were exposed for 28 days to 2 different sub-lethal concentrations of pesticides (malathion, 10 and 20 ppm; hexachlorocyclohexane (HCH), 8 and 16 ppm) separately, and the fifth lot in ordinary tap water was used as control	T3, T4, T3/T4 ratio	-Decrease in plasma T4 level within 4 weeks of exposure to malathion (10 and 20 ppm) -Increase in the plasma level of T4 induced by high concentrations of HCH (16 ppm) -Reducing of plasma T3 and T3/T4 ratio by exposure to HCH but increased by exposure to malathion	-HCH probably acts directly on the thyroid gland, reducing the synthesis and release of thyroid hormones. -Malathion appears to increase the plasma level of T3 either by stimulating the extrathyroid conversion of T4 to T3, or by reducing the excretion of T3
[8]	Exposition of acclimatized *Clarias batrachus* females to sublethal concentrations of malathion (0.007 ml/L) for 96 hours and (0.0035 ml/L) for 16 days during the prespawning and spawning phases of its annual reproductive cycle	T3, T4, T3/T4 ratio, peroxidase activity, extrathyroidal conversion of T4 to T3	At H96 (during the 2 phases): -Lower circulating T3 levels and the T3/T4 ratio -In the pharyngeal thyroid, T4 and peroxidase were high ; T3 and the T3/T4 ratio decreased. -In the posterior kidney, all parameters were reduced -Inhibition of the extrathyroidal conversion of T4 to T3 At D16 (prespawning phase): decrease in T4 and peroxidase in the pharyngeal thyroid with increase in T3 and the T3/T4 ratio At D16 (spawning phase): -Decrease in serum T4 without affecting T3 (increase in the T3/T4 ratio) -In the posterior kidney: significant reduction in T4, T3 and peroxidase and increase in the T3/T4 ratio	-Malathion inhibits the secretion of T4 in the kidney but increases its synthesis in the pharyngeal thyroid -Malathion blocks the extrathyroid conversion of T4 to T3
[9]	-Division of 32 male day-old chicks into 4 groups of 8 each -Oral administration to group 2, 3 and 4 of dimethoate at doses of 2, 4 and 8 mg/Kg/day for 4 weeks. Group 1 served as a control	-T3, T4 -Iodothyronine 5'-monodeiodinase type 1 (D1) activity	-Significant decrease in serum T4 in all treated groups -Significant decrease in serum T3 in the 2 groups receiving 4 and 8 mg/Kg -Significant decrease in 5'-D activity in the 2	-Dimethoate exerts a thyroid inhibiting action even at low doses -Dimethoate inhibits the synthesis and/or release of T4 at the glandular level -Dimethoate inhibits the

			groups receiving the highest doses of pesticides	peripheral conversion of T4 to T3
[10]	-Random division of 32 adult male Swiss mice in 4 groups of 8 each -Intraperitoneal administration to group 2, 3 and 4 of dimethoate at doses of 2, 4 and 8 mg/Kg, respectively, for 30 consecutive days. Group 1 served as control	-Total T3 (TT3), Total T4 (TT4), Thyroid Stimulating Hormone (TSH) -Iodothyronine 5'-monodeiodinase type 1 (D1) activity	-Significant decrease in TT3 -Increase TT4 in groups treated at medium and high doses -Decreased hepatic 5'-DI activity by the two higher doses of dimethoate	Dimethoate-induced alterations in thyroid function are not mediated by the hypothalamus-pituitary-thyroid (HPT) axis, but by changes in the extrathyroid conversion of T4 to T3
[11]	Oral administration to 6 groups of 8 ewes, of empty gelatin capsules or capsules containing chlorpyriphos, trifluralin, lindane or pentachlorophenol twice a week for 43 days. Dimethoate, carbofuran, 2,4-dichlorophenoxyacetic acid or triallate were administered 3 times per week	Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), progesterone, estradiol, T4, cortisol, insulin	OPs have resulted in a marked decrease in T4 concentrations	Several pesticides may influence serum levels of reproductive and metabolic hormones, especially T4
[12]	The litters of 20 rats were reduced to 8 pups each at birth Lactating rats were divided into 2 groups of 10 each: -G1: control group -G2: treated with dimethoate (40 mg/Kg) until D10 post-delivery	Free T3 (FT3), Free T4 (FT4), TSH	-Reduction in the body weight of young exposed rats, attributed to a deficiency in TH -Decrease in plasma FT4, FT3 and thyroid iodine and increase in TSH in mothers and their offspring	Dimethoate can reduce thyroid secretion activity in lactating rats and may even slow body growth
[13]	-Exposition of 20 fish to sublethal concentrations of dimecron in separate tanks containing 200 L of pesticide water. A control group was also kept in similar tanks containing pesticide-free water -6 surviving fish from each of the control and the experimental tanks were used for the survey	T3, T4, cortisol, prolactin, insulin	-Significant reduction in T3 during the different exposure periods -Significant increase of T4 at H6 and D5	Fish adaptively maintain a likely low metabolic rate, as indicated by reduced levels of thyroid hormone (T3), which could be considered beneficial for fish to indirectly reduce the toxic impact of the pesticide
[14]	Division of 7-week-old Sprague-Dawley rats into 4 groups of 12 each, receiving chlorpyriphos-methyl (CPM) at 0, 1, 10 or 100 mg/Kg/day Treatment of parental rats for 2 weeks of pre-breeding and for a maximum of 2 weeks of breeding. Then, exposure of the male rats for an additional 3 weeks and the female rats for an additional 6 weeks of gestation and lactation	-T3, T4, TSH -Estradiol, testosterone, corticosterone, cholesterol	Serum T4 and T3 levels were significantly lower while TSH was higher in female and male F1 rats treated with CPM	CPM induces an anti-androgenic effect and hypothyroidism after long-term exposure *in utero* via the sexual maturation of rats
[15]	Division of 34 male albino rats into 4 groups: G1 (10): 30 mg/Kg Methamidophos (lethal dose) / G2 (7): NaCl G3 (10): 30 mg/Kg methamidophos then 40 mg pralidoxime and atropine to suppress cholinergic signs / G4 (7): NaCl	T3, T4, TSH	G1/G2: decrease in rate of T3, T4, TSH in G1 G3/G4: decrease in rate of T3, T4 and increase in rate of TSH in G3 G1/G3: the rate of T4 was low in G1 and the level T3 was higher in G3. TSH level increased in G3 after treatment	Acute exposure to Methamidophos may cause a hypothyroidism syndrome
[16]	-Pregnant dams were treated with 0, 3, 6 mg/Kg/day of CPF on gestational days 15-18 -After delivery, pups were treated subcutaneously on postnatal days	-T3, T4 -Acetylcholinesterase (AChE)	-In dams at 6 mg/Kg : decreased T4 levels and increased cell height in thyroid -In pups : short-term and	CPF exposure at dose levels not inducing brain AChE inhibition causes thyroid alterations in dams mice and pups

	(PND) 11-14 with: 0, 1, 3 mg/Kg/day of CPF		long-term morphological modifications and biochemical alterations (reduced serum T4 levels) at PND 150 with an apparent higher vulnerability of males	Thyroid may be a sensitive target to CPF developmental exposure possibly leading to long-term effects on thyroid function
[17]	-Male mice are moved into female cages to mate. The mating day has been designated as D0.5 gestation -Between gestation days, the mice were injected subcutaneously with 0, 1 or 5 mg/Kg of CPF	TT3, TT4, Free Thyroxine Index (FTI)	FTI increased significantly in females, but not males	Prenatal exposure to low doses of CPF causes persistent behavioral and hormonal changes in adulthood with sexual selectivity, with females more affected
[18]	CPF was given to developing rats on gestational days 17-20 or postnatal days 1-4, regimens that produce distinctly different, sex-selective effects on neurobehavioral performance	T3, T4	Prenatal regimen produced a significant reduction in brain thyroxine levels from juvenile stages through adulthood Postnatal exposure produced a transient elevation in young adulthood	Although CPF may alter brain thyroid hormone levels, the effect is small, and any potential contribution to neurobehavioral abnormalities remains to be proven
[19]	Male goldfish (*Carassius auratus*) exposed to 0.01, 0.10, and 1.00 mg/L of 40% monocrotophos (MCP)	-TT3, TT4, FT3, FT4 -mRNA expression of indices involved in the hypothalamic–pituitary–thyroid axis (HPT axis)	-Decrease of plasma TT3 levels and TT3 to TT4 ratios, without effect on plasma TT4 levels -Profiles of the changes in the relative abundance of deiodinase (D1, D2 and D3) transcripts in liver, brain and kidneys -Increase in the metabolism of T3, expressed as highly elevated hepatic D1 and D3 mRNA levels -Decrease in plasma FT3 levels correlated with modulation of hepatic transthyretin mRNA expression	The MCP exhibited thyroid-disrupting effects via interference with the HPT axis at multiple potential sites
[20]	Female goldfish (*Carassius auratus*) exposed to 0.01, 0.10, and 1 mg/L of 40% MCP for 21 days in a semi-static exposure system	-TT3, TT4, TT3/TT4 ratio, TSH -Expression profiles of HPT axis-responsive genes, including transthyretin (TTR), deiodinases (d1, d2, and d3), TSHB, thyrotropin releasing hormone (TRH), and corticotrophin-releasing hormone (CRH)	-Decrease of plasma levels of TT3 and ratio TT3/TT4, partly attributed to an increase in the metabolism of T3 in the liver (elevated hepatic d1 and d3 mRNA levels in the MCP treatment groups) -Upregulation of TTR mRNA -The TSH levels were lower in females exposed to 0.01 and 0.10 mg/L MCP, whereas the up-regulation of TSHB mRNA levels was compensated by the decreased plasma TT3 levels	The MCP have the potential to influence several pathways of HPT axis homeostasis in female goldfish
[21]	Male goldfish (*Carassius auratus*) exposed to 0, 4, 40, and 400 µg/L MCP for 2, 4, 8, and 12 days	-T3, T4 -17β-estradiol (E2), testosterone -Transcription profiles of the HPG and HPT genes	-The MCP-induced plasma 17β-estradiol (E2) levels were most associated with alteration of cyp19a transcription,	These finding gave a whole view of the regulations, especially on the cross-talk between sex hormone and

			which was also a potential point indirectly modulated by the MCP-altered thyroid hormones status -The disruption of TH pathways was most related with the effect of MCP on regulation of the hypothalamic-pituitary hormones involved in the thyroid system, and the increased E2 levels might enhance the impact of MCP on HPT axis by modulating hepatic deiodinase expression	thyroid hormone pathways upon exposure to chemicals with unknown direct target *in vivo*

Table 2. Summary table of studies in humans analyzing the OP-thyroid disruption relationship

Study	Methodology	Assessment parameters	Results	Conclusion
[22]	322 married men under investigation for infertility or sterility recruited from January 2000 to April 2003	-TT3, FT4, TSH -Urinary 3,5,6-trichloro-2-pyridinol TCPy (metabolite of chlorpyrifos and chlorpyrifos-methyl) -Urinary 1-napththol 1N (metabolite of carbaryl and naphthalene)	-Association between TCPy and TSH -Suggestive inverse association between TCPy and FT4 -No associations between 1N and thyroid hormones	Environmental exposure to chlorpyrifos, chlorpyrifos-methyl, or its metabolite TCPy may alter thyroid function in human males
[23]	136 male subjects occupationally exposed to OPs, during two agricultural periods (rainy season and dry season)	-TT3, TT4, TSH -Dialkylphosphate (DAP) -Serum p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) -Serum paraoxonas-1 (PON1) activity	Association between the increase of total dimethylphosphate levels (ΣDMP) in the urine and the increase of both TSH and T4 hormones in serum on one side, and the decrease in TT3 serum levels in the other side	Exposure to OPs may increase TSH and T4 serum level's and decrease T3 serum level's, so they may act as endocrine disruptors in humans
[24]	Data of 3249 individuals aged 12 years and over, from the U.S. National Health and Nutrition Examination Surveys (NHANES)	-TT4, TSH -Urinary TCPy	-Association between TCPy and higher T4 in men aged 12 to less than 18 years and 18 to less than 40 years -Association between TCPy and lower TSH in men aged 18 to 40 -Contradictory associations for TSH in men and women aged > 60 years	Exposure to certain OPs or their metabolites may disrupt the HPT axis
[25]	637 pregnant women enrolled from April 2011 to December 2013	-TSH, TT3, TT4, FT3, FT4 -DAP	Positive association between urinary DAP with FT4 levels and negative with TSH	Exposure to OPs may change the thyroid function in pregnant women

Table 3. Summary table of *in vitro* studies analyzing the OP-thyroid disruption relationship

Study	Methodology	Assessment parameters	Results	Conclusion
[26]	-Assessment of the effect on thyroid hormone (TH) function and aryl hydrocarbon receptor (AhR) transactivity -Analyze of 13 pesticides in cell culture: 2-methyl-4-chlorophenoxyacetic acid, terbuthylazine, iodosulfuron-methyl-sodium, mesosulfuron-methyl, metsulfuron-methyl, chlormequat chloride, bitertanol, propiconazole, prothioconazole, mancozeb and its metabolite ethylene thiourea, cypermethrin, tau-fluvalinate and malathion	-GH3 cell proliferation assay (T-screen) -AhR responsive luciferase reporter gene bioassay	Malathion significantly stimulated the growth of GH3 cells compared to the maximum response induced by T3	Pesticides tested can interfere with TH signaling and AhR function *in vitro* and could cause endocrine disruption
[27]	*In vitro* toxicogenomic experiments, generating data verified *in vivo*: -Exposition of PCCl3 cells (immortalized rat thyrocytes) for one week to different doses of ethylene-thiourea (ETU) and chlorpyriphos (CPF) and to their combination -Exposition of mice from conception (GD 0) to CPF and ETU (0.1, 1 and 10 mg/kg/day) and their combination. Molecular and phenotypic thyroid damage were analyzed	-High-throughput RNA-sequencing (RNAseq) data analysis -Ingenuity Pathway Analysis (IPA) -FT4	*In vitro* data revealed specific and common genes / mechanisms of toxicity, controlling the proliferation and survival of thyrocytes. These results were confirmed phenotypically *in vivo* by the reduction of the circulating T4 hormone after long exposure	*In vitro* toxicogenomics is a powerful tool in predicting adverse effects *in vivo*, although not fully recapitulating the biocomplexity of a living animal.

Conflicts of interest

Authors do not declare any conflict of interest.

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