Review Article

Carbamates pesticides induced immunotoxicity and carcinogenicity in human: A review

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\textbf{ABSTRACT}

In the literature, carbamates pesticides (CMs) have been implicated in the increasing prevalence of diseases associated with alterations of the immune response, such as hypersensitivity reactions, some autoimmune diseases and cancers. CMs may initiate, facilitate or exacerbate pathological immune processes, resulting in immunotoxicity by induction of mutations in genes coding for immunoregulatory factors and modifying immune tolerance. In the present study, oxidative stress and inhibition of esterases activities have been introduced as the main mechanisms of CMs induced immune dysregulation. In addition, the evidence on the relationship between CMs pesticide exposure, dysregulation of the immune system and predisposition to different types of cancers are criticized.

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\textbf{Introduction}

The main role of the immune system is to protect all other organ systems from invading pathogens, which include infectious agents and malignant cells, and to eliminate senescent or phenotypically altered cells, such as cancer cells, that are not performing their appropriate functions. Toxicants can be chemical agents, for example, pesticides, respectively, which induce harmful effects. Depending on the dose, a pesticide may range from being essential (necessary for the maintenance of health) to being toxic. When this chemical is stated to be toxic, it does not necessarily mean that it induces the death of cells; a toxic effect may not result in cytotoxicity, but alteration of cell function, leading to a detrimental outcome.

Among the most used worldwide pesticides, there are carbamates pesticides (CMs). These compounds derived from carbamic acid are probably the insecticides with the widest range of biocidal activities. The structure of the biologically active carbamates is displayed in Fig. 1. CMs pesticides are inhibitors of acetylcholinesterase activity (AChE) and therefore, are also able to reversibly inhibit neuropathy target esterase. However, carbamates are not able to “age” the
inhibited enzyme, and therefore, they are not inducers of neuropathy (Lotti, 1992). The main detoxification routes of carbamate insecticides are hydrolysis and oxidation, the hydrolysis by esterases being a most effective detoxification route. Although, the role of esterases in the carbamate detoxification is well understood there are no in-depth toxicological and biochemical studies in mammals on interactions between these enzymes and carbamate insecticides (Lotti, 1992; Lotti and Moretto, 1999).

Extensive research has focused on investigating carbamates toxicity. Indeed, literature, including PubMed, Scopus and Google Scholar was searched to identify authentic articles published in English language during the last 20 years. The main keywords of the search were “carbamate” in combination with each of the terms “immunotoxic”, “immunotoxicity”, and “immune system”. Additional searches included the terms “insecticide”, “herbicide” and “fungicide” in combination with “immune system” with no limitation in the type or date of publication. Besides, additional articles were extracted from the reference lists of the found publications. All publications were growing evidence indicating that carbamates pesticides also have deleterious effects on the immune system (Bernier et al., 1995; Cha et al., 2000; El-Bini Dhouib et al., 2014). Carbamates may potentially play a role in carcinogenesis of various tissues through reduced immune surveillance. However, the specific effects of CMs on immune function remain poorly understood. Therefore, we considered that further investigation of CMs immunotoxicity is warranted and conducted a PubMed search of CMs exposure and cancer immune-related effects. Here, we summarize the known toxicological effects of carbamates pesticides on immune function in humans.

### Mechanism of action of carbamates pesticides on immune cells

Carbamates pesticides interfere with metabolism, signal transduction pathways, structural tissue compartments and cellular structures.

#### Cholinesterase inhibition

The understanding that carbamates insecticides share the same mechanism of action achieved by the inhibition of AChE, a serine hydrolase, and that the serine hydrolase activity is probably linked to several immune functions, gave a clearer picture of the potential immunotoxicity of these compounds (Fukuto, 1990) (Fig. 2).

Banks and Lein (2012) demonstrated that CMs can alter lymphocytic cholinergic signaling through inhibition of acetylcholinesterase (AChE). Indeed, in acute CMs poisoning,
overstimulation of cholinergic receptors by accumulated acetylcholine (Ach) can cause rapid and transient Ca^{2+} signaling, up-regulation of c-Fos expression and interleukin (IL)-2-induced signal transduction in T and B cells as well as triggering inflammatory responses in macrophages (Banks and Lein, 2012). Moreover, CMs can alter esterases activities associated with the cell membrane of immunocytes (Banks and Lein, 2012).

**Oxidative stress**

In most cases, the metabolism of pesticides can cause the generation of toxic metabolites or reactive oxygen species (ROS) (Lasram et al., 2014) (Fig. 2). These reactive intermediates may induce cytolethality through damaging the components of the cell, including lipids, proteins and DNA. Further, CMs-induced oxidative stress can disturb various parts of cellular signaling and oxidative homeostasis through mediating redox signaling and depletion of anti-oxidant reservoirs (Abdollahi et al., 2004; El-Bini Dhouib et al., 2014). Additionally, as an alternative mechanism, CMs-induced oxidative stress may promote carcinogenic mutations via induction of DNA damage (Klaunig and Kamendulis, 2004).

Induction of free radicals, lipid peroxidation and impaired antioxidant status by CMs have been widely studied in humans (Gupta et al., 2001). The studies reported in manufacturing workers accidentally exposed to CMs pesticides are also similar. Vidyasagar et al. (2004) reported an increase of MDA level and superoxide dismutase (SOD) activity in human cases accidentally poisoned by various CMs. Ranjar et al. (2002) also reported an increase in MDA levels and reduced thiols and total antioxidant capacity among manufacturing workers exposed to different doses of CMs pesticides. Thus, acute and chronic exposure of male rats to different doses of carbosulfan, causes the same changes in the redox status in the liver, spleen and thymus (El-Bini Dhouib et al., 2014, 2015).

Oxidative stress was reflected by an increase of lipid peroxidation (LPO). Indeed, increase of LPO has been demonstrated after acute and/ or chronic exposure to other carbamates like benomyl and thiram (Banks and Soliman, 1997; Cereser et al., 2001). Yang and Dettbarn (1996) observed a strong correlation between the accumulation of acetylcholine and the extent of LPO. Also, butyrylcholinesterase (BChE), a cholinesterase enzyme, decreased in spleen of carbosulfan treated rats (El-Bini Dhouib et al., 2014). BChE can be considered as an endogenous scavenger of anticholinesterase compounds; and detoxifies them before they reach to AChE at physiologically important target sites. Some important compounds of carbamates and organophosphorous pesticides were detoxified by BChE (Adresi, 2003). Increased oxidative stress by CMs might be a result of cholinergic hyperactivity or might be due to its direct effect on the production of reactive oxygen and nitrogen species. The mechanism involved in increased production of reactive oxygen species was the inhibition of cytochrome c oxidase (Gupta et al., 2001). It is also reported that CMs induces nitric oxide synthase, which is implicated in the overproduction of superoxide anions (Pou et al., 1992). End products of LPO are believed to be largely responsible for the cytotoxic effects observed in spleen (Pou et al., 1992; El-Bini Dhouib et al., 2014). Therefore, carbamates induced oxidative stress which may contribute to its immunotoxicity as previously reported (Caroleo et al., 1996; Gao et al., 2015).

Free radicals are known to attack unsaturated fatty acid side chains of phospholipids, causing a substantial decrease of these cell membrane components. This effect leads to reduced membrane fluidity (Gao et al., 2015). In physiological conditions, the process underlying lymphocyte interaction with antigens or with other cell subsets requires the integrity of cell membrane. It is possible that free oxygen radicals generated by CMs pesticides could inhibit T-cell function via membrane lipids peroxidation (Gao et al., 2015). El-Bini Dhouib et al. (2014) demonstrated that in the cell system, treatment of lymphocytes with carbosulfan induced a profound decrease in their total and reduced glutathione (GSH) content in rat spleen. Also, the susceptibility of T-cells to lipid peroxidation may be the result of lower levels of intracellular antioxidants, such as reduced GSH and it is important in lymphocyte activation and proliferation. Altogether, many data confirm the hypothesis of immunotoxicity induced by oxidative stress caused by CMs (Handy et al., 2002; El-Bini Dhouib et al., 2014; Singh et al., 2015). It is clear from these studies that the immune system may be a sensitive target for CMs pesticides for which the exact molecular target or mechanism of immunotoxicity was oxidative stress. Hence, immunosuppression induced by carbosulfan and carbofuran may be a consequence of toxic chemical-induced cholinergic stimulation and its effects on immune cells function (Jeon et al., 2001). Moreover, CMs may also act directly or indirectly on lymphoidal cells, immunglobulin metabolism, T/B cell macrophage cooperation and macromolecular biosynthesis responses. Such as, some dithiocarbamates compounds base their immunotoxic potential also on oxidative stress and on inflammation. Indeed, the expression of nuclear factor-κB (NF-κB) indicates that this can be the convergence point of the immune and neuroendocrine pathways.

**Endocrine disruption**

Endocrine disruption refers to mechanism of toxicity that impairs the ability of cells or the organs to communicate hormonally. Because immune system is strictly controlled by hormones released from various organs, endocrine disruptor pesticides, such as CMs pesticides, can profoundly disturb both homeostasis and function of immune system (Mostafalou and Abdollahi, 2013; Mokarizadeh et al., 2015). Since, hypothalamic–pituitary–adrenal axis (HPA axis), a major part of the neuroendocrine system, controls reaction to stress and regulates immune responses, CMs can disturb immune system through dysregulation of gonadotropin-releasing hormone (GnRH) biosynthesis in hypothalamus gland (Corsini et al., 2013). The alteration of immune system may occur directly through altering GnRH-receptor signaling in immune cells or indirectly by alteration in cortisol secretion from adrenal gland. In view of the fact that the adrenal gland induces production of a variety of cytokines, interactions between carbofuran (a carbamate pesticide) and HPA axis may result in change in the cytokine network (Blakley et al., 1999; Zhao et al., 2010; Mokarizadeh et al., 2015).
Immune alterations on humans

The majority of papers on the clinical immunotoxicity of carbamates pesticides described immune alterations in occupational exposed workers or farmers, and accidentally exposed humans. In some instances, these alterations were suggested to be associated with infectious complications.

In workers exposed to CMs, the killing ability of neutrophils has been shown to be significantly abrogated, probably via interference with myeloperoxidase activity (Corsini et al., 2013). A carefully designated epidemiological study conducted in 23 women chronically exposed to aldicarb-contaminated groundwater at level of 16.1 ppb showed persistent changes in the T-cell subset count with a significant increase number of T-CD8+ cells and a decreased T4/T8 ratio compared to a matched control group of 27 unexposed women (Fiore et al., 1986). A significant negative correlation between average daily aldicarb ingestion and T4/T8 ratio values suggested a dose-dependent effect. In vitro lymphocyte stimulation to Candida antigen was increased in exposed women, again with a dose response effect. However, the values were within the normal range of variability. In addition, the lack of similar response with other antigens or mitogens and the small number of tested patients strongly limit the significance of these results. Overall, it has been suggested that CMs modulate immune responses through a series of separate mechanisms including inhibition of serine hydrolases in immune cells, oxidative damage to immune organs and modulation of signal transduction pathways in women (Corsini et al., 2013) and in children (Jones et al., 2014).

In addition, numerous studies have been shown on the effects of CMs pesticides on immune system (Viala et al., 1996). Moreover, in vivo and in vitro studies have shown that, CMs can affect immune responses including antibody production, IL-2 production and T-cell proliferation, autoantibody production, changes in Th1 and Th2 cytokine production, NK, LAK and TC cells inhibition via different mechanisms (Li et al., 2002; Li, 2007). On the other hand, systemic poisoning with carbaryl can result immune suppression, increasing the risk of allergic responses against allergens such as dust mites coming from domestic waste due to inappropriate immune responses (Gholam et al., 2014).

Role of CMs-induced immunosuppression in relationship to carcinogenicity

The idea that the immune system might recognize and destroy tumor cells was conceived more than 100 years ago. An overwhelming amount of data from animal models, together with compelling data from human patients, indicate that a functional cancer immnosurveillance process exists that works to prevent outgrowth of many types of primary and transplanted tumors (Hanahan and Weinberg, 2011). It has also become clear, however, that the immune system can also facilitate tumor progression. The recognition that immunity plays a dual role in the complex interactions between tumors and the host prompted a refinement of the cancer immnosurveillance hypothesis into one termed cancer immunoediting (Dunn et al., 2004). Chemical-induced immunotoxicity is comprised of three phases; this process is collectively denoted the three E’s of cancer immunoediting: elimination, equilibrium and escape:

1. elimination: cancer immune surveillance,
2. equilibrium: a phase of tumor dormancy where tumor cells and immunity enter into a dynamic equilibrium that keeps tumor expansion in check,
3. escape where tumor cells emerge that either display reduced immunogenicity or engage a large number of possible immunosuppressive mechanisms to attenuate antitumor immune responses leading to the appearance of progressively growing tumors. One can envisage that.

Factors that tumor exploit to avoid immune responses

Immunosuppression in the tumor induced by carbamates pesticides, mediated by CD4+CD25+ FoxP3+ regulatory T cells (Tregs), or other types of suppressive cells, seems to be a major mechanism of tumor immune escape and can be a crucial hurdle for tumor immunotherapy (Whalen et al., 2003).

It is well established that another fundamental mechanism by which tumors evade immune surveillance is by down-modulating antigen processing machinery affecting essentially the major histocompatibility complex (MHC) I pathway (Mahajan et al., 2007). Thus, expression of tumor antigen is downregulated, which can lead to enhanced tumor incidence and metastasis because cytotoxic T lymphocyte (CTL) can no

Fig. 3 – Tumor growth and immune response.
longer recognize target antigens on the tumor cells (Corsini et al., 2013).

As alluded to above, tumors can evade immune surveillance by crippling CTL functionality after chronic exposure to carbofuran via production of several immune suppressive cytokines, either by the cancer cells or by the noncancerous cells present in the tumor microenvironment, especially including immune cells and epithelial cells. TGF-β is a chief mediator of this activity (Jeon et al., 2001). In addition, tumor necrosis factor (TNF)-α, IL-1, IL-6, colony stimulating factor (CSF)-1, IL-8, IL-10, and IFN-γ can also significantly contribute to cancer growth (Lind et al., 2004).

In the other hand, tumors are also known to evade immune attack by shifting the balance from Th1 to Th2 (immune deviation) in a TGF-β and IL-10-dependent manner (Jeon et al., 2001).

In the end, apoptosis is another fundamental mechanism by which tumors evade immune surveillance. A number of studies have shown that cancer cells delete tumor specific CTLs through apoptosis (Yoon et al., 2001).

The different factors governing tumor growth and immune evasion strategies are briefly outlined in Fig. 3.

**Conclusion, recommendations and future directions**

This review summarized the researches about the immunotoxicity of carbamates pesticides on human:

1) CMs cause immunosuppression and present in the tumor microenvironment from the very earliest stages of tumor formation. Many of the chapters in this review delineate some of the cellular and molecular mechanisms by which carbamates pesticides alter the immune system.

2) An understanding of the normal development of the cellular components of the immune system, the manner by which they interact, and the known parameters by which their structure and function can be modified is necessary for the pursuit of investigations into how environmental agents alter our health by changing our immune system.

3) CMs may contribute to tumorigenesis either through interference with immune surveillance or by innate immune dysfunction leading to chronic inflammation. Nonetheless, since CMs pesticides can cause cancer through a number of non-immunologic pathways (such as: genetic damage), the responsibility of immune dysregulation in onset of cancers linked to CMs pesticides is not absolute.

4) Oxidative stress is the main mechanism by which CMs pesticides can increase susceptibility to immunotoxicity and infectious diseases.

5) In spite of many ongoing studies, the exact immune toxicity of most pesticides is still unclear. Additionally, more epidemiological and experimental studies should be conducted to reveal the exact relationship between the level of exposure and toxic effect.

6) Moreover, inconsistencies in epidemiological findings, possibly due to differences in dose, sampling, genetic background, and environmental/nutritional factors, indicate need for larger participant numbers and diverse ethnic populations. Due to differential effects of exposures, populations having low, intermediate and high exposure should be evaluated to better understand dose-dependent relationships.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**


