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Genetic Toxicological Profile of Carbofuran and Pirimicarb Carbamic Insecticides

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1. Introduction

It's well known that the pesticide usages in agriculture have led increase in food production worldwide. Although the benefits of conventional agricultural practices have been immense, they utilize levels of pesticides and fertilizers that can result in a negative impact on the environment (WHO, 1988). Only for the 2006-2007, the total world pesticide amount employed was approximately 5.2 billion pounds (www.epa.gov). Their application is still the most effective and accepted method for the plant and animal protection from a large number of pests, being the environment consequently and inevitably exposed to these chemicals. Herbicides accounted for the largest portion of total use, followed by other pesticides, like insecticides and fungicides (www.epa.gov). The goal in pesticide investigation and development is identifying the specificity of action of a pesticide toward the organisms it is supposed to kill (Cantelli-Forti et al., 1993). Only the target organisms should be affected by the application of the product. However, because pesticides are designed and selected for their biological activity, toxicity on non-target organisms frequently remains a significant potential risk (Cantelli-Forti et al., 1993). The benefits in using pesticides must be weighed against their deleterious effects on human health, biological interactions with non-target organisms, pesticide resistance and/or accumulation of these chemicals in the environment (WHO, 1988). Pesticides are high volume, widely used environmental chemicals and there is continuous debate concerning their probable role in both acute and chronic human health effects (Cantelli-Forti et al., 1993; Hodgson & Levi, 1996). Among the potential risk effects of agricultural chemicals, carcinogenesis is of special concern. The genetic toxicities of pesticides have been determined by numerous factors like their biological accumulation or degradation in the environment, their metabolism in humans, and their action in cellular components such as DNA, RNA and proteins (Shirasu, 1975). It seems essential the determination of the genotoxic risks of these pesticides before they are used in agriculture. Therefore, the carcinogenic and mutagenic potential of a large amount of pesticides has been the object of an extensive and wide investigation (WHO, 1990). These results have great predictive value for the carcinogenicity of several pesticides (IARC, 1987). The International Agency for Research on Cancer (IARC) has reviewed the potential carcinogenicity of a wide range of insecticides, fungicides, herbicides and other similar compounds. Fifty-six pesticides have been classified with carcinogenic potential in different laboratory animals (IARC, 2003). Among them, and as a brief example, chemicals compounds as phenoxy acid herbicides, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), lindane,

methoxychlor, toxaphene, and some organophosphates have been reported with a carcinogenic potential in human studies (IARC, 2003).

Numerous well known pesticides have been tested in a wide variety of mutagenicity as well as DNA, chromosomal, and cellular damage endpoints (IARC, 2003). Several investigations have been reported positive associations between exposure and pesticide risk (Shirasu, 1975; Bolognesi et al., 1993, 2009, 2011; Pavanello & Clonfero, 2000; Bolognesi, 2003; Clark & Snedeker, 2005; Castillo-Cadena et al., 2006).

2. Carbamic insecticides

The carbamates are chemicals mainly used in agriculture as insecticides, fungicides, herbicides, nematocides, and/or sprout inhibitors (IARC, 1976). These chemicals are part of the large group of synthetic pesticides that have been developed, produced, and used on a large scale within the last 50 years. Additionally, they are used as biocides for industrial or other applications as household products including gardens and homes (IARC, 1976).

During the last decades, considerable amounts of pesticides belonging to the class of carbamates have been released into the environment. Humans may be exposed to carbamates through food and drinking water around residences, schools, and commercial buildings, among others (IARC, 1976). Consequently, carbamates are potentially harmful to the health of different kinds of organisms (EPA, 2004). Among all classes of pesticides, carbamates are most commonly used compounds because organophosphates and organochlorines are extremely toxic and possess delayed neurotoxic effects (Hour et al., 1998). They share with organophosphates the ability to inhibit cholinesterase enzymes and therefore share similar symptomatology throughout acute and chronic exposures. Likewise, exposure can occur by several routes in the same individual due to multiple uses, and there is likely to be additive toxicity with simultaneous exposure to organophosphates (IARC, 1976).

The *N*-methyl carbamates are a group of closely related pesticides employed in homes, gardens and agriculture that may affect the functioning of the nervous system (EPA, 2007). Toxicological characteristics of the *N*-methyl carbamates involve maximal cholinesterase enzyme inhibition followed by a rapid recovery, typically from minutes to hours (EPA, 2007). Several compounds namely aldicarb, carbaryl, carbofuran, formetanate HCl, methiocarb, methomyl, oxamyl, pirimicarb, propoxur, and thiodicarb are included as members of the *N*-methyl carbamate class (EPA, 2007).

3. Carbofuran. Genotoxicity and cytotoxicity profiles

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate; CASRN: 1563-66-2) is one of the most widely granular employed *N*-methyl carbamate esters with both contact and systemic activity. Carbofuran is a derivative of carbamic acid being its chemical structure formula shown in Fig. 1.

Carbofuran is a relatively unstable compound that breaks down in the environment within weeks or months (www.inchem.org). It is registered on a variety of agricultural uses to control soil-dwelling and foliar-feeding insects, mites and nematodes on a variety of field, fruit, forage, grain, seed, and fiber crops (EPA, 2006). Carbofuran is a systemic, broad spectrum insecticide and nematocide registered *N*-methyl carbamate for control of soil and foliar pests. It has been reported for 2006 that nearly one million pounds of carbofuran was

applied worldwide (EPA, 2006). The most sensitive and appropriate effect associated with the use of carbofuran is its toxicity following acute exposure (HSDB, 2011). On the basis of its acute toxicity, it has been classified as a highly hazardous member (class Ib) by WHO (2009) and highly toxic compound (category I) by EPA (2006) based on its potency by the oral and inhalation exposure routes. In spite of the recommendation and regulation proposed by the United States Environmental Protection Agency (EPA) concerning the use of this carbamate within the United States of America, its application has been recently cancelled all over the Northern country by the same organization since 2009 (www.epa.gov). However, the contamination of environment with this compound can by far occur, particularly taking into consideration those countries where it is still in use and the probability of long-term low dose exposure becomes increased. Due to its extensive employment in agriculture and household, contamination of food, water and air has become serious and undesirable health problem for humans, animals and wildlife. Large quantities of this carbamate are particularly applied to different environments worldwide.

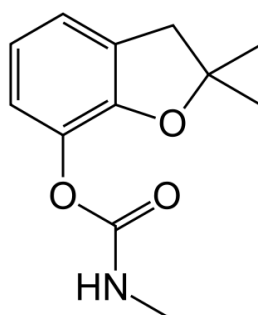


Fig. 1. Chemical structure of carbofuran. Source: INCHEM (www.inchem.org).

Metabolism of carbofuran has been extensively studied in plants and animals (Dorough & Casida, 1964; Metcalf et al., 1968). In mammals, it reversibly inhibits acetylcholinesterase by carbamylation as well as others non-specific serine-containing enzymes, such as carboxylesterases and butyrylcholinesterases (Gupta, 1994). This results in accumulation of acetylcholine at nerve synapses and myoneural junctions leading to cholinergic signs and causing toxic effects (Karczmar, 1998). Epidemiological studies suggested that exposure to carbofuran may be associated with increased risk of gastrointestinal, neurological, cardiac dysfunction, and retinal degeneration (Cole et al., 1998; Kamel et al., 2000; Peter & Cherian, 2000). Carbofuran represents an acute poison when absorbed into the gastrointestinal tract by inhalation of dust and spray mist and minimally poison through the intact skin contact (Gupta, 1994). In summary, carbofuran is reported to be teratogenic, embryotoxic and highly toxic to mammals (Gupta, 1994; WHO-FAO, 2004, 2009; WHO, 2009).

Genotoxicity and cytotoxicity studies have been conducted with this *N*-methyl carbamate member using several end-points on different cellular systems. A summary of the results reported so far is presented in Table 1.

The compound produced both conflicting and inconclusive results in mutagenicity tests varying according to either the end-point assessed (WHO, 1988, 2000-2002, 2009; WHO-FAO, 2004, 2009). When mutagenic activity was assessed in bacterial systems either positive or negative results have been reported. Carbofuran has been found to be non-mutagenic in *Salmonella typhimurium* since negative or weak positive response were observed in the number of mitotic recombinants regardless of the presence or absence of a rat liver

metabolic activation system (Blevins et al., 1977a; Gentile et al., 1982; Waters et al., 1982; Haworth & Lawlor, 1983; Hour et al., 1998; Yoon et al., 2001). These results indicate that carbofuran cannot be considered mutagenic in bacterial systems. However, it was active in *Salmonella typhimurium* TA1538 and TA98 strains in the presence or absence of S9 metabolic system (Gentile et al., 1982; Moriya et al., 1983; Hour et al., 1998). Whereas the insecticide did not induce reverse mutations in *Escherichia coli* (Simmon, 1979), it has been claimed as a relatively weaker mutagen with the repair defective Ames *Escherichia coli* K-12 test (Saxena et al., 1997). Similarly, positive results have been found after exposure in *Vibrio fischeri* regardless the absence or presence of S9 metabolic system (Canna-Michaelidou & Nicolaou, 1996). When DNA damage and repair assays were performed, carbofuran was also negative in both *Escherichia coli* and *Bacillus subtilis* bacterial systems (SRI, 1979). Similar negative results were also found after carbofuran exposure in *Saccharomyces cerevisiae* mitotic recombination assay (Simmon, 1979).

The mammalian *in vitro* gene mutation assay systems generated results consistent with the microbial gene mutation assays, although they were generally more responsive. When a mammalian cell system was employed for mutagenic screening, carbofuran was found to be positive in V79 cells (Wojciechowski et al., 1982). Similar results were reported for the cell mutation assay in mouse lymphoma L5178 cells (Kirby, 1983a, b). Unscheduled DNA synthesis was monitored in human fibroblasts and primary rat hepatocytes following treatment with the insecticide with and without S9 fraction. Both negative and positive results were obtained for the same endpoint in human primary fibroblasts regardless of the presence or absence of a rat liver metabolic activation system (Simmon, 1979; Gentile et al., 1982) but negative results were obtained in primary rat hepatocyte cultures (SRI, 1979). Single-strand breaks detected by alkaline comet assay were induced in *in vitro* human peripheral lymphocytes (Das et al., 2003; Naravaneni & Jamil, 2005). The induction of DNA fragmentation on human skin fibroblasts have been found to be enhanced after *in vitro* carbofuran treatment (Blevins et al., 1977b).

As opposed to mutation assays that detect specific gene defects, the chromosomal assays evaluate the structure of the whole chromosome. Five studies of carbofuran have evaluated the induction of sister chromatid exchanges in mammalian cell cultures. In one of the first studies, carbofuran was negative in Chinese hamster ovary cells regardless of the presence or absence of S9 fraction (Thilagar, 1983b). However, other authors reported positive results for the same cell system (Gentile et al., 1982; Thilagar, 1983c; Lin et al., 2007; Soloneski et al., 2008) as well as human lymphocytes (Georgian et al., 1985). Similarly, the effects on chromosomal structure following exposure to carbofuran were investigated in Chinese hamster ovary and primary human lymphocytes cells. While carbofuran did not induce *in vitro* chromosome damage in Chinese hamster ovary cells with or without metabolic system activation (Thilagar, 1983a), positive results were reported to occur not only in the same cellular system (Lin et al., 2007) but also in human lymphocytes *in vitro* (Pilinskaia & Stepanova, 1984; Das et al., 2003). However, inconclusive response for this endpoint has been also reported to occur in the latter system after carbofuran exposure (Naravaneni & Jamil, 2005). Positive results have been also reported for the ability of carbofuran to induce micronuclei in both Chinese hamster ovary cells and human lymphocytes *in vitro* with and without S9 metabolic fraction (Soloneski et al., 2008; Mladinic et al., 2009).

Several assays have been developed to assess the ability of carbofuran to cause cytotoxic effects on different cellular systems. Negative response was observed in both *Escherichia coli* and *Bacillus subtilis* bacterial systems (Simmon, 1979). When the analysis of cell-cycle

End-point/Test System	Concentration ^a	Results	References
<i>In vitro</i> assays			
Ames test			
<i>Salmonella typhimurium</i> , S9 +/-	100 – 10 000 µg/plate	+/-	Blevins et al., 1977a; Waters et al., 1982; Haworth & Lawlor, 1983; Yoon et al., 2001
<i>Salmonella lactam</i> assay, S9 +/-	1 – 10 000 µg/plate	-	Hour et al., 1998
	0.1 – 100 µg/plate	+	Gentile et al., 1982; Moriya et al., 1983; Hour et al., 1998
Pol A reverse mutation			
<i>Escherichia coli</i> (WP ₂), S9 +/-	1 – 5 000 µg/plate	-	Simmon, 1979
<i>Escherichia coli</i> (K-12)	1 – 5 000 µg/plate	+	Saxena et al., 1997
Mutatox test			
<i>Vibrio fischeri</i> (M169), S9 +/-	175 µg/plate	+	Canna-Michaelidou & Nicolaou, 1996
DNA damage and repair			
<i>Escherichia coli</i> (W3110-p3478)	0 – 5 mg/6-mm disk	-	SRI, 1979
<i>Bacillus subtilis</i> (H17-M45)	0 – 5 mg/6-mm disk	-	SRI, 1979
Mitotic recombination			
<i>Saccharomyces cerevisiae</i> (D3), S9 +/-	1 – 50 mg/ml	-	Simmon, 1979
Gene mutation assay			
V79 cells	NA	+	Wojciechowski et al., 1982
Cell mutation tk locus			
Mouse lymphoma L5178 Y cells, S9 +/-	16 – 1 780 µg/ml	+/-	Kirby, 1983a, b
UDS	0.1 – 1 000 µg/ml	-	Simmon, 1979
Human fibroblasts (WI-38), S9 +/-	0.1 – 1 000 µg/ml	+	Gentile et al., 1982
Human lung fibroblasts	0 – 100 µg/ml	-	SRI, 1979
Primary rat hepatocytes			
Alkaline comet assay			
HL	NA	+	Naravaneni & Jamil, 2005
	0.5 – 4.0 µM	+	Das et al., 2003
DNA fragmentation analysis			
Human skin fibroblasts	NA	+	Blevins et al., 1977b
SCE assay			
CHO cells, S9 +/-	12.5 – 312.5 µg/ml	-	Thilagar, 1983b
CHO cells, S9 +/-	12.5 – 2 500 µg/ml	+	Thilagar, 1983c
CHO-K1 cells	5 – 100 µg/ml	+	Gentile et al., 1982; Soloneski et al., 2008
CHO-W8 cells	0.04 – 0.32 µg/ml	+	Lin et al., 2007
HL	NA	+	Georgian et al., 1985

Chromosomal aberrations			
CHO cells, S9 +/-	50 – 2 500 µg/ml	-	Thilagar, 1983a
CHO-W8 cells	0.04 – 0.32 µg/ml	+	Lin et al., 2007
HL	NA	+/-	Naravaneni & Jamil, 2005
HL	100 – 300 µg/ml	+	Pilinskaia & Stepanova, 1984
HL ^b	NA	+	Das et al., 2003
Micronuclei assay			
CHO-K1 cells	10 – 100 µg/ml	+	Soloneski et al., 2008
HL, S9 +/-	0.008 µg/ml	+	Mladinic et al., 2009
Growth inhibition			
<i>Escherichia coli</i>	1 – 500 mg/ml	-	Simmon, 1979
<i>Bacillus subtilis</i>	1 – 500 mg/ml	-	Simmon, 1979
Alteration in CCP			
CHO-W8 cells	0.04 – 0.32 µg/ml	-	Lin et al., 2007
CHO-K1 cells	50 – 100 µg/ml	+	Soloneski et al., 2008
CHL cells	30 µM	-	Yoon et al., 2001
Brain tubulin assembly assay			
Porcine cells	100 – 2 000 µmol/l	+	Stehrer-Schmid & Wolf, 1995
Cell viability			
CHL cells	30 µM	-	Yoon et al., 2001
CHO-K1 cells	50 – 100 µg/ml	+	Soloneski et al., 2008
Apoptosis			
CHL cells	30 µM	-	Yoon et al., 2001
Mouse brain microvascular endothelial cells	3 – 30 µM	-	Jung et al., 2003
Rat cortical cells	500 µM	+	Kim et al., 2004
<i>In vivo</i> assays			
Reverse mutation			
<i>Zea mays</i>	NA	-	Gentile et al., 1982
Sex-linked recessive lethal test			
<i>Drosophila melanogaster</i>	0 – 10 ppm	-	DeGraff, 1983; Gee, 1983
Dominant -lethal mutagenicity			
Mice	0.025 – 0.5 mg/Kg/day	-	FMC, 1971
UDS			
Rat hepatocytes	5 – 10 ppm	-	Valencia, 1981; 1983
Alkaline comet assay			
Mouse peripheral lymphocytes	0.1 – 0.4 mg/Kg bw	-	Zhou et al., 2005
HL*	NA	+	Castillo-Cadena et al., 2006

SCE			
Mouse peripheral lymphocytes	NA	+	Gentile et al., 1982
Rat	NA	+	Aly, 1998
Chromosomal aberrations			
<i>Allium cepa</i>	20 – 80 ppm	+	Saxena et al., 2010
<i>Allium sativum</i>	20 – 80 ppm	+	Saxena et al., 2010
<i>Drosophila melanogaster</i>	NA	-	Woodruff et al., 1983
Mouse bone marrow cells	3.8 – 1.9 (for 4 days) mg/Kg bw	+	Chauhan et al., 2000
Mouse bone marrow cells	0.1 – 1.0 mg/Kg bw	-	Pilinskaia & Stepanova, 1984
Rat bone marrow cells	0.6 – 10 mg/Kg bw	-	Putman, 1983b, a
HL ^b	NA	+	Zeljezic et al., 2009
Micronuclei			
Mouse peripheral lymphocytes	0.1 – 0.4 mg/Kg bw	-	Zhou et al., 2005
Mouse bone marrow cells	5.7 – 1.9 (for 4 days)	-	Chauhan et al., 2000
Alteration in CCP			
<i>Allium cepa</i>	20 – 80 ppm	+	Saxena et al., 2010

UDS, unscheduled DNA synthesis; SCE, sister chromatid exchange; HL, human lymphocytes; CCP, cell-cycle proliferation; NA, data not available.

Table 1. Evaluation of carbofuran-induced genotoxicity and cytotoxicity on different target systems. ^a, expressed as reported by authors; ^b, exposed to pesticide mixture containing carbofuran; *, from agricultural workers occupationally exposed to carbofuran.

progression on mammalian cells was studied, carbofuran gave negative results in Chinese hamster ovary cells and lung fibroblasts (Yoon et al., 2001; Lin et al., 2007). On the other hand, Soloneski and co-workers (2008) reported a delay in the cell-cycle progression of Chinese hamster ovary cells after the insecticide treatment. Carbofuran was tested *in vitro* in the porcine brain tubulin assembly assay for detecting whether the chemical can be considered as a microtubule poison and an aneuploidy agent. A dose-dependent reduction in the degree of polymerization of tubulins was reported in porcine cells after *in vitro* treatment (Stehrer-Schmid & Wolf, 1995). Controversial results were reported for the cell viability assay in mammalian cells, e.g., Chinese hamster lung and ovary cells after the exposure (Yoon et al., 2001; Soloneski et al., 2008). Finally, whereas carbofuran-induced apoptosis has been reported in rat cortical cells (Kim et al., 2004), negative results have been also observed in mouse brain microvascular endothelial cells and Chinese lung fibroblasts (Yoon et al., 2001; Jung et al., 2003). Similar end-points for both genotoxicity and cytotoxicity were also applied in *in vivo* systems. Carbofuran has been reported as a non inducer agent of mutations in plants cells, at least in *Zea mays* (Gentile et al., 1982), in the *Drosophila melanogaster* sex-linked recessive lethal test (DeGraff, 1983; Gee, 1983), and in the mice dominant-lethal mutagenicity test (FMC, 1971). Negative results have been obtained for the induction of unscheduled DNA synthesis in primary rat hepatocytes (Valencia, 1981, 1983). Controversial observations have been reported for the induction of DNA single-strand breaks assayed by the alkaline comet assay. Positive results were reported in circulating erythrocytes from occupationally exposed workers (Castillo-Cadena et al., 2006) whereas no induction was observed in mouse peripheral lymphocytes exposed *in vivo* (Zhou et al., 2005). It should be noted that the former positive

results could not be totally committed to carbofuran but to other pesticides, since the cohort of donors included in the study was exposed to a panel of other pesticides. Several reports were able to reveal that carbofuran increased the frequency of sister chromatid exchanges in mammalian cells from mouse and rats exposed *in vivo* (Gentile et al., 1982; Aly, 1998), and chromosomal aberrations in plants from *Allium* (Saxena et al., 2010), and mammals including occupationally exposed workers (Putman, 1983a, b; Pilinskaia & Stepanova, 1984; Chauhan et al., 2000; Zeljezic et al., 2009) but not in insects (Woodruff et al., 1983) as well as in rodent cells (Putman, 1983a, b; Pilinskaia & Stepanova, 1984). When the micronuclei induction end-point was employed in mouse, no induction was found either in bone marrow cells (Chauhan et al., 2000) or circulating lymphocytes (Zhou et al., 2005). Finally, alterations in the progression of the cell-cycle were reported to occur after carbofuran exposure in plants when the *Allium cepa* model was employed (Saxena et al., 2010).

4. Pirimicarb. Genotoxicity and cytotoxicity profiles

Pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate, CASRN: 23103-98-2) is a dimethylcarbamate insecticide member with both contact and systemic activity. Similar to carbofuran, pirimicarb is a derivative of carbamic acid being its chemical structure formula shown in Fig. 2.

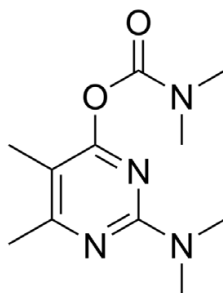


Fig. 2. Chemical structure of pirimicarb. Source: INCHEM (www.inchem.org)

Based on its acute toxicity, pirimicarb has been classified as a moderately hazardous compound (class II) by WHO (http://www.who.int/ipcs/publications/pesticides_hazard/en/) and slightly to moderately toxic (category II-III) by EPA (1974a). Among carbamate pesticides, pirimicarb is registered as a fast-acting selective aphicide mostly used in a broad range of crops, including cereals, sugar beet, potatoes, fruit, and vegetables, and is relatively non-toxic to beneficial predators, parasites, and bees (WHO-FAO, 2004, 2009). It acts by contact, translaminar, vapor, and systemic action. Its mode of action is inhibiting acetylcholinesterase activity (WHO-FAO, 2004, 2009).

Available information on the genotoxic and cytotoxic properties of pirimicarb is limited and inconsistent. Only few data are available in the literature (WHO-FAO, 2004, 2009). Genotoxicity and cytotoxicity studies have been conducted with this carbamate using several end-points on different cellular systems. A summary of the results reported so far is presented in Table 2.

Pirimicarb has been generally recognized as non-genotoxic in bacteria, yeast and fungi as well as in mammalian cells (EPA, 1974b). It has been reported to be non-mutagenic in *Salmonella typhimurium* when the Ames reversion mutagenicity test for the TA1535, TA1538, TA98, and TA100 strains after S9 metabolic activation has been used (Trueman, 1980; Callander, 1995). Furthermore, similar situation was observed in both *Escherichia coli* and *Aspergillus nidulans* when the reverse mutation assay or recessive lethal gene mutation test

End-point/System	Concentration ^a	Results	References
<i>In vitro</i> assays			
Ames test			
<i>Salmonella typhimurium</i> , S9 +/-	2 500 µg/plate	-	Trueman, 1980
	5 000 µg/plate	-	Callander, 1995
Pol A reverse mutation			
<i>Escherichia coli</i>	5 000 µg/plate	-	Callander, 1995
Recessive lethal gene mutation			
<i>Aspergillus nidulans</i>	NA	-	Käfer et al., 1982
Cell mutation tk locus			
Mouse lymphoma L5178 Y cells	1 400 mg/ml - S9	-	Clay, 1996
	100 mg/ml + S9	+	Clay, 1996
Alkaline comet			
HL	50 - 500 µg/ml	+	Ündeger & Basaran, 2005
SCE assay			
CHO-K1 cells	100 - 200 µg/ml	+	Soloneski & Larramendy, 2010
Chromosomal aberrations			
CHO-K1 cells	10 - 300 µg/ml	+	Soloneski & Larramendy, 2010
HL, S9 +/-	500 µg/ml	-	Wildgoose et al., 1987
Alteration in CCP			
CHO-K1 cells	100 - 300 µg/ml	+	Soloneski & Larramendy, 2010
<i>In vivo</i> assays			
Eye mosaic system w/w+			
<i>Drosophila melanogaster</i>	NA	+	Aguirrezabalaga et al., 1994
Dominant lethal mutation			
Mice	20 mg/Kg bw	-	McGregor, 1974
UDS			
Rat liver cells	200 mg/Kg bw	-	Kennelly, 1990
Chromosomal aberrations			
Rat bone marrow cells	50/-100 mg/Kg bw	-	Anderson et al., 1980
HL*	NA	+	Pilinskaia, 1982
Micronuclei assay			
<i>Cnesterodon decemmaculatus</i>	50 - 157 mg/L	+	Vera Candioti et al., 2010b
<i>Rhinella arenarum</i>	80 - 250 mg/L	+	Vera Candioti et al., 2010a
Rat bone marrow cells	69.3 mg/Kg bw	-	Jones & Howard, 1989

Table 2. Evaluation of Pirimicarb-induced genotoxicity and cytotoxicity on different target systems. ^a, expressed as reported by authors; UDS, unscheduled DNA synthesis; SCE, sister chromatid exchange; HL, human lymphocytes; CCP, cell-cycle proliferation; NA, data not available.

were respectively applied (Käfer et al., 1982; Callander, 1995). Negative and positive results were obtained for the induction of mutagenicity in mouse lymphoma L5178Y cells regardless of the presence or absence of a rat liver metabolic activation system (Clay, 1996). Furthermore, the induction of DNA single strand breaks, estimated by the alkaline comet assay, was evaluated revealing positive results in human lymphocytes exposed *in vitro* to pirimicarb (Ündeger & Basaran, 2005). Similar positive results were found when the sister chromatid exchange assay was performed in Chinese hamster ovary cells (Soloneski & Larramendy, 2010). Although a significant increase in chromosomal aberrations has been reported in Chinese hamster ovary cells after pirimicarb exposure (Soloneski & Larramendy, 2010), Wildgoose and coworkers (1987) observed negative results in human lymphocytes with or without S9 metabolic activation. Finally, the induction of alterations in the cell-cycle progression in Chinese hamster ovary cells was reported to occur after *in vitro* exposure to pirimicarb (Soloneski & Larramendy, 2010).

In *in vivo* genotoxic and cytotoxic studies, pirimicarb was able to induce different types of lesions. It has been reported the ability of the insecticide to give positive results by using the eye mosaic system *white/white⁺* (*w/w⁺*) somatic mutation and recombination test (SMART) when *Drosophila melanogaster* was employed as experimental model (Aguirrezabalaga et al., 1994). However, McGregor and co-workers (1974) reported negative results in mice when the dominant lethal mutation assay was performed. Similar negative results were found by Kenelly (1990) using the unscheduled DNA synthesis in rat liver cells. At the chromosomal level, pirimicarb did not induce chromosomal alterations in bone marrow cells of Wistar male rats after oral administration (Anderson et al., 1980). Contrarily, Pilinskaia (1982) observed a significant increase of chromosomal aberrations in the peripheral blood lymphocytes from occupational workers after pirimicarb exposure. Finally, when the micronuclei induction end-point was employed, positive results were reported in erythrocytes of the fish *Cnesterodon decemmaculatus* and *Rhinella arenarum* tadpoles by Vera Candioti and collaborators (Vera Candioti et al., 2010a, b). Lastly, when a mammal model was employed for the micronuclei detection, Jones and Howard (1989) found negative results in rat bone marrow cells.

5. Comparison of the genotoxicity and cytotoxicity of carbofuran and pirimicarb and some Argentinean technical formulations

One of the goals of our research group is to compare the genotoxic and cytotoxic effects exerted by the pesticide active ingredients (Pestanal®, Riedel-de Haën, Germany) and their technical formulations commonly used in Argentina on vertebrate cells both *in vitro* and *in vivo*.

The evaluation was performed using end-points for genotoxicity [Sister Chromatid Exchange, Chromosome Aberration, and Micronuclei frequencies] and cytotoxicity [Mitotic Index, Cell Viability, Proliferative Rate Index, Erythroblasts/Erythrocytes Ratio, 3(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Neutral Red assays] (Soloneski et al., 2008; Soloneski & Larramendy, 2010; Vera Candioti et al., 2010a, b).

We comparatively evaluated the genotoxic and cytotoxic *in vitro* effects on mammalian Chinese hamster ovary cells induced by the pure insecticide carbofuran and its commercial formulation Furadan® (47% carbofuran, FMC Argentina S.A., Buenos Aires, Argentina). Similarly, the genotoxic and cytotoxic *in vitro-in vivo* effects induced by the pure insecticide pirimicarb and its commercial formulation Aficida® (50% pirimicarb, Syngenta Agro S.A.,

Buenos Aires, Argentina) on mammalian Chinese hamster ovary cells as well as circulating erythrocytes of the fish *Cnesterodon decemmaculatus* and the amphibian *Rhinella arenarum* tadpoles were also estimated.

A summary of the results obtained is presented in Fig. 3. The figure clearly reveals that all compounds assayed were able to inflict damage at chromosomal and cellular level regardless of the cellular system used as target.

We observed that carbofuran/Furadan® and pirimicarb/Aficida® caused SCEs on mammalian cells indicating that they have a clastogenic activity (Fig. 3A). It has been suggested that at the chromosomal level, the induction of SCEs is a reliable indicator for the screening of clastogens, since the bioassay is more sensitive than the analysis of clastogen-induced chromosomal aberrations (Palitti et al., 1982). The results also demonstrate the ability of pirimicarb/Aficida® to induce DNA damage quali- and quantitative analyzed by the frequency of chromosomal aberrations (Fig. 3B). Furthermore, a putative clastogenic/aneugenic activity exerted *in vitro* by carbofuran/Furadan® and *in vivo* by pirimicarb/Aficida® was also demonstrated by the ability of the pesticide-induced micronuclei (Fig. 3C). The analysis of the proliferative replication (Fig. 3D) and the mitotic indexes (Fig. 3E) demonstrated that both carbofuran/Furadan® and pirimicarb/Aficida® were able to delay the cell-cycle progression as well as to exert a marked reduction of the cellular mitotic activity on mammalian cells *in vitro*. Besides, carbofuran/Furadan® and pirimicarb/Aficida® were able to induced a clear cellular cytotoxicity. This deleterious effect was estimated by a loss of lysosomal activity (indicated by a decrease in the uptake of neutral red), as well as alteration in energy metabolism (measured by mitochondrial succinic dehydrogenase activity in the MTT assay), as clearly revealed in insecticides-treated Chinese hamster ovary cells (Fig. 3F).

Overall, the results revealed, depending upon the endpoint employed, that the damage induced by the commercial formulations of both insecticides is, in general, greater than that produced by the pure pesticides (Fig. 3). Unfortunately, the identity of the components present in the excipient formulations was not made available by the manufacturers. These final remarks are in accord with previous observations not only reported by us but also by other research groups indicating the presence of xenobiotics within the composition of the commercial formulations with genotoxic and cytotoxic effects (David, 1982; Lin & Garry, 2000; Soloneski et al., 2001, 2002, 2003, 2008; González et al., 2007a, b, 2009; Elsik et al., 2008; Molinari et al., 2009; Soloneski & Larramendy, 2010). These observations highlight that, in agriculture, agrochemicals are generally not used as a single active ingredient but as part of a complex commercial formulations. Thus, both the workers as well as non-target organisms are exposed to the simultaneous action of the active ingredient and a variety of other chemical/s contained in the formulated product. Hence, risk assessment must also consider additional geno-cytotoxic effects caused by the excipient/s.

Finally, the results highlight that a whole knowledge of the toxic effect/s of the active ingredient of a pesticide is not enough in biomonitoring studies as well as that agrochemical/s toxic effect/s should be evaluated according to the commercial formulation available in market. Furthermore, the deleterious effect/s of the excipient/s present within the commercial formulation should be neither discarded nor underestimated. The importance of further studies on this type of pesticide in order to achieve a complete knowledge on its genetic toxicology seems to be, then, more than evident.

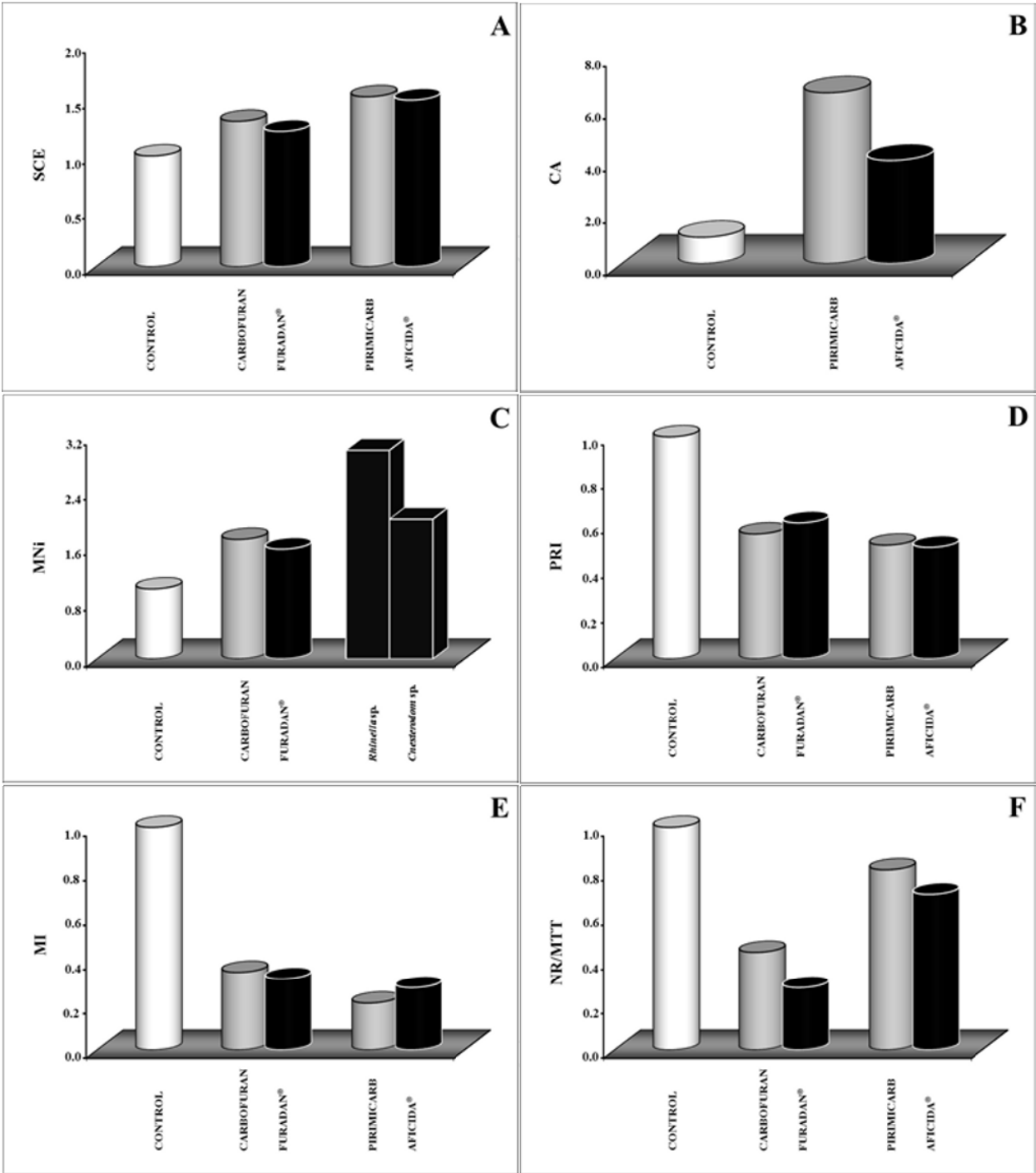


Fig. 3. Comparative genotoxicity and cytotoxicity effects induced by carbofuran and pirimicarb pure herbicides Pestanal® (grey) and their technical formulations Furadan® and Aficida® (black) commonly used in Argentina on mammalian Chinese hamster ovary cells (cylinders) and *in vivo* piscine and amphibian erythrocytes (bars). Results are expressed as fold-time values over control data. Evaluation was performed using end-points for genotoxicity [Sister Chromatid Exchanges (A), Chromosome Aberrations (B), Micronuclei (C)] and cytotoxicity [Proliferative Rate Index (D), Mitotic Index (E), 3(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Neutral Red (NR) (F)].

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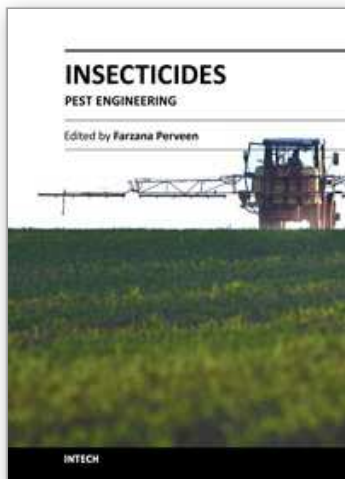
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Insecticides - Pest Engineering

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This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by *Bacillus thuringiensis*, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of *Aedes aegypti*, etc. Section C describes bioactive natural products from sapindacea, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of *Anopheles gambiae* P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarb carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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