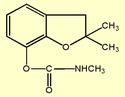
#940

Carbofuran: Toxicity Profile and Non-Dietary Risk Evaluation

Andrew L. Rubin[1], Sheryl Beauvais[2] and Keith Pfeifer[1]

[1]Medical Toxicology & [2]Worker Health & Safety Branches, Dept. of Pesticide Regulation, Cal-EPA, Sacramento, CA

INTRODUCTION



- Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; MW, 221.26) is a broad spectrum, systemic insecticide, acaricide and nematicide. It is used in many crop systems against a large number of target species.
- As a member of the carbamate class of pesticides, the action of carbofuran (CF) is based on its ability to inhibit acetylcholinesterase (AChE) in the nervous system and motor endplates of the target species. CF's toxicity in mammalian systems may also be based on this property.
- This human health risk assessment was conducted under the California SB-950 program in light of indications from rodent studies of CF-induced chronic and reproductive toxicity, as well as mutagenicity. CF is also known to be severely acutely toxic.

HAZARD IDENTIFICATION & DOSE-RESPONSE

Acute toxicity, critical study: WARF (1978)

- CF administered daily by gavage, gestation days 6-15 inclusive
- 24 pregnant CD rats/dose @ 0, 0.1, 0.3 & 1.0 mg/kg/day
- Dams sacrificed on gestation day 20

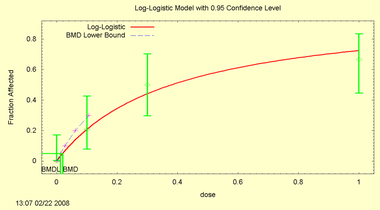
Results:
- 1 high-dose female death (gestation day 9)
- Several toxic signs at the high dose, one sign (chewing behavior) at all doses (Table 1)
- Chewing behavior (an acute response) subjected to benchmark dose analysis; LED_{05} / ED_{05} = 0.01 mg/kg / 0.02 mg/kg (Figure 1)
- The LED_{05} of 0.01 mg/kg was used to evaluate acute risk from the oral, dermal and inhalation routes

Table 1. Toxic signs in CD rats following acute gavage exposure to CF, gd 6-15 (WARF, 1978)

	Carbofuran, mg/kg/day			
	0	0.1	0.3	1.0
N	24	24	24	24
Lethargy	0	0	5*	2
Lacrimation	0	0	0	2
Pale eyes	0	0	0	4
Salivation	0	0	0	5*
Rough coat	3	0	4	6
Trembling	0	0	0	14**
Convulsions	0	0	0	9*
Chewing motions	0	5*	12**	16**

*, **; p<0.05, 0.01, respectively

Figure 1. Incidence of chewing behavior in pregnant CD rats following gavage dosing with carbofuran (WARF, 1978): Log-logistic curve fitting with LED_{05} and ED_{05} intercepts



Acute toxicity, main support study: FMC (2002)

- CF administered as a single dose by gavage
- 8-9 CD rats/sex/dose @ 0, 0.5 & 1.0 mg/kg/day
- Jugular vein cannulated to facilitate blood sampling over the 8-hr time period

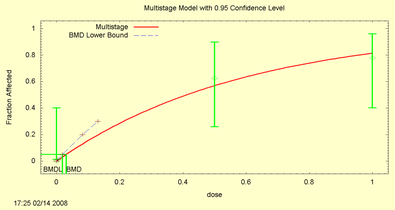
Results:
- No deaths
- Clinical signs at both doses: teeth grinding (chewing behavior) and tremors (Table 2)
- RBC ChE suppressed at both doses; less suppression of plasma ChE (Table 2)
- Teeth grinding in females was subjected to benchmark dose analysis; LED_{05} / ED_{05} = 0.02 mg/kg / 0.03 mg/kg (Figure 2)

Table 2. Clinical signs in CD rats following acute gavage exposure to CF, (FMC, 2002)

	Carbofuran, mg/kg		
	0	0.5	1.0
Teeth grinding [1]			
Males	0/8	3/9	4/9 [2]
Females	0/8	5/8*	7/9**
Body tremors [1]			
Males	0/8	0/9	4/9 [2]
Females	0/8	1/8	8/9**
RBC ChE [3]			
Males	747±59 (n=7)	550±10 (n=9)*	510±15 (n=9)*
Females	747±18 (n=6)	587±15 (n=8)*	542±15 (n=9)*
Plasma ChE [4]			
Males	164±16 (n=7)	140±14 (n=9)	151±12 (n=9)
Remales	316±31 (n=6)	316±29 (n=29)	268±22 (n=9)

*, **: p<0.05, 0.01 (Fisher exact statistics were executed by DPR.)
[1] Observations were limited to one occurrence per animal.
[2] p=0.053
[3] mU/ml of packed RBC; the values expressed are the mean minimum activities over the 8-hr period
[4] mU/ml of plasma; the values expressed are the mean minimum activities over the 8-hr period

Figure 2. Incidence of teeth grinding in female CD rats following a single gavage dose with carbofuran (FMC, 2002): Mststage curve fitting with LED_{05} and ED_{05} intercepts



Subchronic / chronic toxicity, critical study: Pant *et al.* (1995)

- CF administered by gavage for 60 days (5 days/wk)
- 10 male Druckrey rats/dose @ 0, 0.1, 0.2, 0.4 & 0.8 mg/kg/day
- At sacrifice, reproductive organs were weighed, testes were removed for histopathology & testicular enzyme assays, and epididymal sperm were counted and evaluated for motility & abnormalities

Results:
- Seven high dose deaths (time of death not stated)
- High-dose survivors showed lethargy & imbalance (overt toxicity not noted at other doses)
- Changes in reproductive organ wts., sperm motility, sperm & Sertoli cell abnormalities, testicular enzyme levels ≥0.2 mg/kg/day (Table 3)
- NOEL = 0.1 mg/kg/day
- Several other studies provided evidence for testicular toxicity

Table 3. Testicular and body weight effects resulting from subchronic gavage exposure to carbofuran in Druckrey rats (Pant *et al.*, 1995)

	Carbofuran, mg/kg/day				
	0 (n=10)	0.1 (n=10)	0.2 (n=10)	0.4 (n=10)	0.8 (n=3) [6]
Body wt. @ 60 days (g)	216	210	188	156	146
Organ weights					
Testes					
Absolute (g)	2.50	2.50	2.40	2.40	2.36
Relative [2]	1.15	1.20	1.20	1.48**	1.60*
Epididmydes					
Absolute (g)	0.80	0.78	0.50*	0.49*	0.43*
Relative [2]	0.38	0.37	0.30*	0.32*	0.27*
Seminal vesicles					
Absolute (g)	0.18	0.17	0.09*	0.08*	0.05*
Relative [2]	0.08	0.08	0.05*	0.05*	0.03*
Ventral prostate					
Absolute (g)	0.11	0.11	0.09*	0.05*	0.03*
Relative [2]	0.05	0.05	0.04*	0.03*	0.01*
Coagulating gland					
Absolute (g)	0.05	0.05	0.02*	0.02*	0.01*
Relative [2]	0.02	0.02	0.01*	0.01*	0.01*
Sperm motility (%)	85.0	83.7	63.7*	51.2*	36.6*
Sperm count ($x10^7$)	9.0	8.0	5.0*	4.0*	3.0*
Total sperm abnormalities (%) [3]	10.48	10.80	22.25*	33.80*	54.60*
Testicular enzymes [4]					
G6PDG [5]	15.0	14.7	8.5*	6.5*	4.2*
δ-GT	28.9	30.5	60.0*	65.5*	72.0*
SDH	5.2	5.4	3.2*	3.2*	2.3*
LDH	365.0	372.0	501.0*	501.0*	703.0*

*p<0.05
[1] Body weights were estimated by the reviewer from inspection of a bar graph in the study report. Statistical analysis was not performed.
[2] Relative to body weights.
[3] The values for abnormal sperm are the sums of the percentages of various individual abnormal morphotypes. Morphotypes that were statistically significantly increased included detached head, curved neck, bent neck, curved tail, bent tail, round tail, looped tail, and signet tail.
[4] Enzyme units were not provided in the text. However, units were expressed as nmol/min/mg for LDH and δ-GT in a follow-up paper. Since similar activities were obtained for these enzymes, it is assumed that nmol/min/mg applies in this case as well.
[5] G6PDH, glucose-6-phosphate dehydrogenase; SDH, sorbitol dehydrogenase; δ-GT, δ-glutamyl dehydrogenase; LDH, lactate dehydrogenase.
[6] Only 3, as opposed to 10, rats were examined at the high dose due to the 70% mortality at that dose.

EXPOSURE ASSESSMENT & RISK CHARACTERIZ'N

Occupational exposure, handlers:
- Due to a dearth of studies, the Pesticide Handler Exposure Database (PHED), a non-chemical-specific program, was used to estimate most handler exposures
- The only exception to this was for dip/slurry applicators, which used equations in the Risk Assessment Guidance for Superfund, Part-E (RAGS-E) for dermal exposures and air saturation concentration for inhalation exposures (Table 4)

Results:
- Acute, subchronic and chronic exposure estimates for most occupational tasks resulted in dermal and inhalation margins of exposure (MOEs) less than a health-protective standard of 100 (with many MOEs<1)

Resident / bystander exposures (ambient and application site):
- Ambient air measurements, defined as those occurring in urban areas and in areas that are far from application sites, were made at five sites in Imperial County and under two different wind conditions in Sacramento County (Table 5)
- Application site air measurements, defined as those occurring near a field undergoing a specific pesticide application, were conducted at monitoring stations sited 20 meters to all sides of a 70-acre alfalfa field. CF was applied for 1 hr at a rate of 1 lb/acre using ground-boom equipment (Table 5)

Results:
- Acute, subchronic and chronic exposure estimates from ambient air monitoring resulted in MOEs higher than the health-protective standard of 100
- Acute application site exposure estimates resulted in 1-hr MOEs of 18 (infants) and 101 (adults), and 24-hr MOEs of 22 (infants) and 46 (adults)

Table 4. Exposure dosages and margins of exposure (MOEs) for workers handling carbofuran: short-term (acute), seasonal (subchronic) and annual (chronic) scenarios by the dermal and inhalation routes

Work task	Acute ADD [1] (mg/kg)		SADD [2] (mg/kg/day)		AADD [3] (mg/kg/day)	
	Dermal	Inhalation	Dermal	Inhalation	Dermal	Inhalation
Groundboom						
Mixer / loader	0.221	0.003	0.055	0.001	0.009	0.0001
(MOE)	(<1)	(3)	(2)	(100)	(11)	(1000)
Applicator	0.291	0.027	0.073	0.007	0.012	0.001
(MOE)	(<1)	(<1)	(1)	(14)	(8)	(100)
Aerial						
Mixer / loader	0.552	0.008	0.138	0.002	0.023	0.0003
(MOE)	(<1)	(1)	(<1)	(50)	(4)	(333)
Applicator	6.36	0.041	2.12	0.016	0.354	0.003
(MOE)	(<1)	(<1)	(<1)	(6)	(<1)	(33)
Flagger	1.09	0.011	0.271	0.003	0.045	0.001
(MOE)	(<1)	(<1)	(<1)	(33)	(2)	(100)
Chemigation						
Mixer / loader	1.16	0.015	0.290	0.004	0.072	0.001
(MOE)	(<1)	(<1)	(<1)	(25)	(1)	(100)
Low pressure handwand						
M/L/A	0.002	0.00005	0.0006	0.00002	0.0001	0.00001
(MOE)	(5)	(200)	(167)	(5000)	(1000)	(10,000)
Dip/slurry						
Mixer / loader	0.002	0.00003	na [9]	na	na	na
(MOE)	(5)	(333)				
Applicator	1.29	0.001	na	na	na	na
(MOE)	(<1)	(10)				
Fieldworker reentry						
Cotton scouts	0.007	na	0.0009	na	0.0001	na
(MOE)	(1)		(111)		(1000)	
Alfalfa scouts	0.099	na	0.070	na	0.012	na
(MOE)	(<1)		(1)		(8)	
Potato scouts	0.016	na	0.010	na	0.002	na
(MOE)	(1)		(10)		(50)	

Note: Dermal and inhalation exposures were calculated from surrogate data using the PHED database and software (DPR, 2006).
[1] Acute Absorbed Daily Dosage (acute ADD) is an upper-bound estimate calculated from the short-term exposure (µg/lb AI). The application rate was set to the maximum on the product labels, which varied for each scenario; acres treated per day also varied by scenario.
Acute ADD = [(acute exposure) x (absorption) x (acres treated / day) x (application rate)] / 70 kg bw
The acute exposure values and acres treated / day are provided in the EAD (DPR, 2006). Calculations assumed dermal absorption = 50%, body weight = 70 kg & inhalation absorption = 100%
[2] Seasonal Average Daily Dosage (SADD) is a 90% upper confidence estimate calculated from the long-term exposure estimate (µg/lb AI). The application rate was set to the maximum rate on product labels, which varied for each scenario; acres treated per day varied by scenario. These are found in the EAD (DPR, 2006). Calculation assumptions were the same as in footnote "1".
SADD = [(long-term exposure) x (absorption) x (acres treated / day) x (application rate)] / 70 kg bw
[3] Annual Average Daily Dosage *(AADD) = SADD x (annual use months per year) (12 months/year).* Annual use estimates varied for each scenario and can be found in the EAD (DPR, 2006).

Table 5. Exposure dosages and margins of exposure (MOEs) for residents and bystanders under ambient and application site conditions

Site	Acute ADD [1] (mg/kg)		SADD [2] (mg/kg/day)		AADD [3] (mg/kg/day)	
	Infants	Adults	Infants	Adults	Infants	Adults
Ambient monitoring:						
Imperial County						
Site C	0.007	0.003	na[4]	na	na	na
(MOE)	(1429)	(3333)				
Site M	0.019	0.010	0.008	0.004	0.001	0.001
(MOE)	(526)	(1000)	(12,500)	(25,000)	(100,000)	(100,000)
Site EC	0.006	0.003	na	na	na	na
(MOE)	(1667)	(3333)				
Site H	0.004	0.002	na	na	na	na
(MOE)	(2500)	(5000)				
Site PM	0.070	0.034	0.020	0.010	0.003	0.002
(MOE)	(143)	(294)	(5000)	(10,000)	(33,333)	(50,000)
Sacramento County						
South winds	0.0014	0.0007	0.0004	0.0002	0.0001	0.00007
(MOE)	(7143)	(14,286)	(250,000)	(500,000)	($1x10^6$)	($1.4x10^6$)
North winds	0.0016	0.0008	0.0005	0.0003	0.0002	0.0001
(MOE)	(6250)	(12,500)	(200,000)	(333,333)	(500,000)	($1x10^6$)
Application site monitoring:						
1-hour	0.550	0.099	na	na	na	na
(MOE)	(18)	(101)				
24-hour	0.454	0.216				
(MOE)	(22)	(46)				

[1] For ambient monitoring, Acute Absorbed Daily Dosage (µg/kg/day) = (95% upper bound air concentration) x (inhalation rate). Calculations assumed infant inhalation rate = 0.59 m^3/kg/day, adult inhalation rate = 0.28 m^3/kg/day & inhalation absorption = 100%.
For 1-hr application site monitoring, *Acute ADD = (1-hr air concentration) x (1-hr inhalation rate).* Calculations assumed heavy 1-hr activity, thus infant inhalation rate = 0.25 m^3/kg/hr & adult inhalation rate = 0.045 m^3/kg/hr.
For 24-hr application site monitoring, *Acute ADD = (TWA air concentration) x (24-hr inhalation rate)*
[2] *Seasonal Average Daily Dosage (SADD) = (mean air concentration) x (inhalation rate).*
[3] *Annual Average Daily Dosage (AADD) = SADD x (# of high-use months / 12 months per year)*
[4] na, not applicable; seasonal and annual exposure estimates were not done at sites with no detects (Site H) or one detect (Sites C and EC); application site exposures were not considered to have seasonal or annual components.

CONCLUSIONS

- Based on low MOE values (which, in turn, were based on very low critical LED_{05}s and NOELs, as well as on relatively high occupational and application site exposure estimates) CF as currently used may present short-term, seasonal and/or annual health risk to agricultural workers and to bystanders near fields under treatment.
- CF does not present a health risk to individuals under ambient exposure conditions.

REFERENCES

DPR. 2006. Estimation of Exposure of Persons in California to Pesticide Products that Contain Carbofuran. Document #HS-1803 (authors: S. Beauvais and J. Johnson), Worker Health and Safety Branch, Dept. of Pesticide Regulation, Cal-EPA. January 6, 2006

FMC. 2002. The toxicokinetics of peripheral cholinesterase inhibition from orally administered carbofuran in adult male and female CD rats. FMC study #A2001-5379. DPR Vol. #254-184, Record #203585

Pant, N., A.K. Prasad, S.C. Srivastava, R. Shankar and S.P. Srivastava. 1995. Effect of oral administration of carbofuran on male reproductive system of rat. *Human & Experimental Toxicol.* 14:889-894

WARF. 1978. Teratogenicity of carbofuran in rats. WARF Institute, Inc., Madison, WI. No study date provided. WARF #T-730. DPR Volume #254-138, Record #87711

Note*: DPR's full Risk Characterization Document for carbofuran may be found at: *http://medtox/docs/pubs/RCDs/carbofuran.pdf