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Serum levels and half-life of carbaryl in buffalo calves after subchronic exposure: implications for withdrwal times

Najeeb Jawad, Rajdeep Kaur, Suresh K. Sharma, Simrat P. S. Saini*

Department of Pharmacology and Toxicology
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana
*spalsaini@yahoo.com

Abstract

Carbaryl is recommended for use on fodders crops in India, but absolutely no work has been done on its toxicokinetics aspect in any species consuming these forage crops, including buffalo species. Since the toxicokinetics serves as a basis for recommending safe withdrawal period of any substance, the aim of this study was to compute important parameters of carbaryl after its subchronic exposure at recommended dose in order to serve as a guideline with regards to observation of meat withdrawal times in buffaloes exposed to carbaryl. Carbaryl was given at the dose rate of 1 mg/kg daily for 105 consecutive days and blood samples of each animal were collected, followed for extraction of carbaryl in serum. Carbaryl produced a gradual accumulation in serum of buffalo calves after oral exposure. There was variation in serum carbaryl levels from 3.31 ± 0.28 to 3.46±0.61 ppm through day 15 to day 105 of exposure, respectively. The area under the curve (AUC) was computed to be 363.0 ± 10.1μg.ml^{-1}.day^{-1}. Volume of distribution at steady state (Vd_{ss}) was found to be 0.162 ± 0.017 ml.kg^{-1}. The overall elimination rate constant (K_{el}) and mean residential time (MRT) were calculated to be 0.017± 0.003 day^{-1} and 59.1 ± 7.8 days, respectively. Based on mean serum levels of carbaryl, the elimination half life ($t_{1/2}\beta$) of carbaryl in buffalo calves was calculated to be 67.37 days. Carbaryl had a long half-life in in buffalo calves. This finding will serve as a guideline with regards to observation of meat withdrawal times in buffaloes exposed to carbaryl. This is very important since carbaryl is recommended for spraying over fodder crops to control pests. However further studies are needed to verify this by measuring actual tissue residue levels of carbaryl at regular time intervals after exposure.

Keywords: Carbaryl, Buffalo, Half-life, Serum, Chromatography
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Introduction

Development of the carbamate insecticides was a major breakthrough in use of pesticide in agriculture industry. Carbaryl is a broad spectrum, general purpose carbamate pesticide used chiefly as an insecticide. It is used worldwide as a substitute for some organochlorine pesticides (1) and also on a variety of fodders (maize, sorghum) for controlling insects like shoot fly and maize borer (2). Although considered quite safe and used extensively, carbaryl exerts adverse effects such as gonadal malfunction (3), estrous cycle disruption (4), embryotoxic, teratogenic (5) and genotoxic (6) properties.

Although, various chromatographic methods of detection of some carbamates have been established in humans for plasma (7), there is no well established method for detection of carabaryl in buffalo calves. The present study was designed to standardize a simple method of detection of carbaryl in serum of buffalo calves and to find its half life in buffalo calves. Absolutely no work has been done on this important aspect of carbaryl; therefore this study will serve as a guideline with regards to observation of meat withdrawal times in buffaloes exposed to carbaryl, since carbaryl is recommended for use even on fodders crops.

* *Author for correspondence*

Materials and Methods

Pesticide

Carbaryl (Sevin ®, 50% DP) pesticide was commercially obtained from Bayer Crop Science Ltd. Mumbai. All other chemical, standards and reagents used in this study were of analytical grade and obtained from reputed companies. The dose given was calculated based on recommendations made in Punjab Agricultural University Package of Practices for Kharif Crops (2).

Animals

Eight healthy male buffalo calves, of 6-10 months age were randomly divided into two groups of four animals each. Group I served as control where as the group II was the treatment group to whom carbaryl was administered orally by drenching at the dose rate of 1 mg/kg/day daily in water for consecutive 90 days. Serum samples of each animals were collected on 0, 15, 30, 45, 60, 75, 90 and 105th days of treatment.

Sample Extraction

The method developed by Frias and coworkers (8) was followed for extraction of carbaryl in serum. After equilibration at room temperature for 15 min, methanol (1.0 ml) was added to 2 ml of serum and the tube vortexed for 2 min. The analytes were extracted with n-hexane-diethyl ether (1:1 v/v, 5.0 ml); vortexed for 2 min and then centrifuged at 2000 rpm for 2 min. The top organic layer was drawn into a clean centrifuge tube. The process was repeated twice. The combined organic phase was concentrated to 2 ml for clean up.

Chromatographic Assay

Clean-up of pesticide in serum sample was performed by column chromatography using florisil (1g), packed into a glass column in between two layers of anhydrous sodium sulphate (0.5 g). The column was pre-washed with hexane and after that the concentrated sample extracts were added to the column. The extract tubes were rinsed five times, initially with 10 ml of 100% hexane and followed by 6%, 15%, 50% of diethyl ether in hexane and finally with 100% diethyl ether . The resultant elute was concentrated to 2 ml and stored in labeled glass stoppered vials for analysis in GLC (gas liquid chromatography). Carbaryl present in cleaned up extracts was estimated in GLC (Clarus 500 (Perkin Elmer)), using nitrogen phosphorous detector (NPD). [Instrumental operating conditions: (1) column: Elite-17ms, Length-30m, id-0.32, DF=0.25, (2) Temp.0 to 300/320°C, (3) Oven temperature programme: Initial temperature: 170 ^{0}C for 5 min; 10^{o}C/min to 220^{o}C and hold for 3 min; 5^{o}C/min to 240^{o}C and hold for 13 min. (4) Detector Ignition: 0.5 – 1 mV, (4) Gas flow rate: Hydrogen: 1ml/min; Air: 100ml/min.The cleaned up extract measuring 5 µl was injected in GLC. Total Chrome software on PC was used for integration and computation of signals. Carbaryl peak was identified and quantified by comparison of the retention time and peak area of the sample chromatographs with those of the carbaryl standard run under the same operating conditions. The standards were run before and after each analysis.

Serum Half-life of Carbaryl

The elimination rate constant (β) was calculated by the method of Gibaldi and Perrier (9). The elimination half-life was computed using the method described by Srivastava and Bal (10).

Results

On analysis using gas chromatography, the retention time of carbaryl was found to be 13.6 min (Figure 1). The efficiency of a GC column increased rapidly with decreasing particle diameter of the packing. The pressure difference required to maintain the given flow rate of carrier gas, however, varied inversely as the square of the particle diameter.

As shown in Table 1, there was a gradual increase of the concentration of serum carbaryl residue from 0th to 90th day of exposure from 3.31 ± 0.28 to 4.37 ± 0.57 ppm, respectively. Thereafter, there was a decreasing trend of concentration till 105th day (3.46±0.61 ppm).

Table 1. Serum concentration of carbaryl in buffalo calves after sub-chronic carbaryl exposure at the dose rate of 1mg/kg/day

Time (Days)	Control	Treatment
0	ND	ND
15	ND	3.31 ± 0.28
30	ND	3.23 ± 0.27
45	ND	3.62 ± 0.36
60	ND	3.21 ± 0.22
75	ND	4.71 ± 0.32
90	ND	4.37 ± 0.57
105	ND	3.46±0.61

Values given are expressed as µg/ml (ppm) and represent Mean ± SE of 4 animals unless otherwise stated. ND stands for not detected.

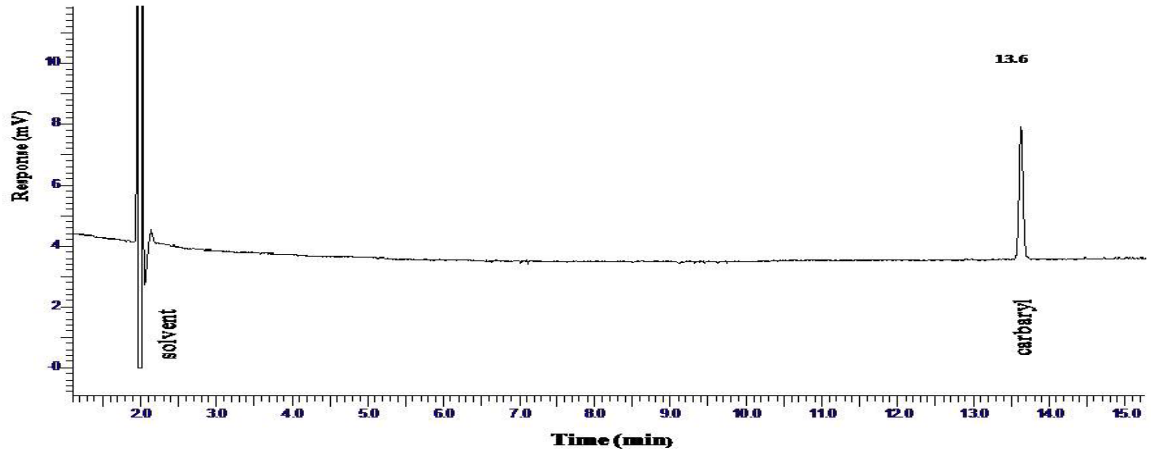


Figure 1. Chromatogram showing peak and retention time of carbaryl in Gas Chromatography.

The area under the curve (AUC) was computed to be 363.0 ± 10.1μg.ml^{-1}.day whereas area under the mean curve (AUMC) was 21454.1± 113.5 μg.ml^{-1}.day^2. The volume of distribution at steady state (Vd_{ss}) was found to be 0.162 ± 0.017 ml.kg^{-1}. The overall elimination rate constant (K_{el}) and mean residential time (MRT) were calculated to be 0.017± 0.003 day^{-1} and 59.1 ± 7.8 days, respectively. Based on mean serum levels of carbaryl, the elimination half life ($t_{1/2}\beta$) of carbaryl in buffalo calves was calculated to be 67.37 days.

Discussion

The gas chromatographic technique used in the present study to quantify carbaryl was highly efficient. The retention time of carbaryl (13.6 min) depends upon its distribution constant which in turn is related to the chemical nature of the stationary phase. In the chromatogram, the carbaryl peak was sharp with no overlapping with any other peak, which depicts the accuracy of method, adequate sensitivity, good stability, reproducibility and a linear response to carbaryl that extended over several orders of magnitude. The ideal situation is to have sharp peaks that are resolved. The longer a substance stays in the column it will cause the peaks to widen. Lengthening the column is a way to improve the separation of different species in the column. A column usually needs to remain at a constant temperature to remain efficient (11). Contaminants, if present, are revealed by the appearance of additional peaks; the areas under these peaks provide rough estimates of the extent of contamination. Absence of additional peaks showed that the purification process used was highly effective with no contaminants.

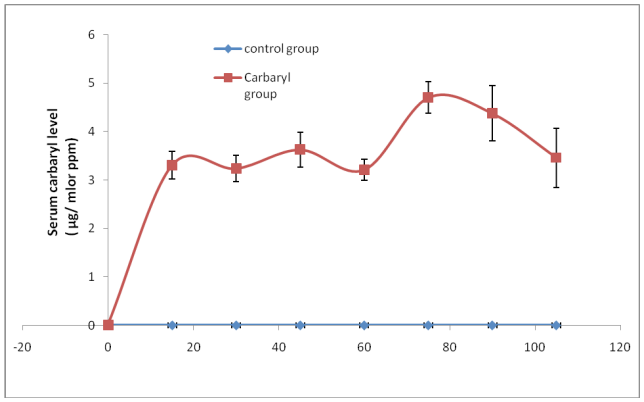


Figure 2. Serum concentration of carbaryl in buffalo calves after sub-chronic carbaryl exposure at the dose of 1mg/kg/day.

As shown in Table 1, there was a gradual increase of the concentration of serum carbaryl residue from 0th to 90th day of exposure from 3.31 ± 0.28 to 4.37 ± 0.57 ppm, respectively. Thereafter, there was a decreasing trend of concentration till 105th day (3.46±0.61 ppm). Coming to the serum levels of carbaryl, on its continuous drenching, residues of carbaryl could be detected in sera of buffaloes. Gradual elevation seen in serum levels upto 90 days of exposure could be due the accumulation after repeated exposure to pesticide and the decrease in later periods could be attributed to the adaptation and induction of metabolism or excretion by the body. The metabolic activities of human liver microsomes and human

cytochrome P450 (CYP) isoforms towards carbaryl were previously investigated and are suggestive of faster metabolism of carbaryl *in vitro* (12). Major metabolites of carbaryl identified in that study were 5-hydroxycarbaryl, 4-hydroxycarbaryl and carbaryl methylol. CYP 1A1 and 1A2 had the greatest ability to form 5-hydroxycarbaryl, while CYP 3A4 and 1A1 were most active in generation of 4-hydroxycarbaryl. The production of carbaryl methylol was primarily the result of metabolism by CYP 2B6.

Table 2. Toxicokinetic parameters of carbaryl in buffalo calves after its sub-chronic exposure at the dose rate of 1mg/kg/day

Parameter	Unit	Mean ± SEM
Area under the curve (AUC)	$\mu g.ml^{-1}.day$	363.0 ± 10.1
Area under the mean curve (AUMC)	$\mu g.ml^{-1}.day^{2}$	21454.1± 113.5
Mean residential time (MRT)	days	59.1 ± 7.8
Overall elimination rate constant (K_{el})	day^{-1}	0.017± 0.003
Elimination half-life ($t_{1/2}\beta$)	days	67.37 ± 9.0
Volume of distribution at steady state (Vd_{ss})	$ml.kg^{-1}$	0.162 ± 0.017
Total body clearance (Cl_b)	$ml.kg^{-1}.day^{-1}$	0.0028 ± 0.0003

Values given are Mean ± SEM of 4 animals unless otherwise stated.

Absolutely, no literature is available on the toxicokinetics of carbaryl in any species. In the present investigation, based on mean serum levels of carbaryl, the pertinent toxicokinetic parameters were calculated in buffalo species using non-compartmental analysis (NCA). NCA, which utilize application of the trapezoidal rule for measurements of the area under the plasma concentration-time curve, is generally the preferred method to use in pharmacokinetics since it requires fewer assumptions than model-based approaches (13). Volume of distribution at steady state (Vd_{ss}) was found to be 0.162 ± 0.017 $ml.kg^{-1}$.The high value of volume of distribution clearly indicates that the pesticide is concentrated in the tissues. Many other pesticides also have high volume of distribution (14). Later in the distribution phase, a xenobiotic distributes to different tissues depending upon its tissue partition coefficient although initially the distribution depends mainly on the blood flow. The overall elimination rate constant (K_{el}) and mean residential time (MRT) were calculated to be 0.017± 0.003 day^{-1} and 59.1 ± 7.8 days, respectively. Both these parameters indicate slower elimination and very high persistence of carbaryl in the body of buffalo calves, and are consistent with its long elimination half-life ($t_{1/2}\beta$ was 67.37 days). This long serum half life of carbaryl could be due to deposition of carbaryl into various tissues of calves, like the adipose tissue and muscles. Some reports in rats (15) have shown carbaryl to be tightly bound mainly to albumin and partly to globulins; this could be another contributing factor for the long half-life observed in buffalo calves. But further studies are needed to confirm this by measuring the tissue concentrations of carbaryl in various body fluids and tissues. This is important from Public Health point of view since no data are available regarding the meat or milk withdrawal times to be followed after exposure of buffalo species to carbaryl, which is expected to be high keeping in view the fact that carbaryl is recommended for spraying even over fodder crops (2).

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Conflict of Interest: None Declared

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