Use of Cholinesterase Activity in Monitoring Organophosphate Pesticide Exposure of Cattle Produced in Tropical Areas

Violeta T. Pardío,* Nelly Ibarra, and Miguel A. Rodríguez

Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, Apartado Postal 1380, Veracruz, Veracruz, México 91700

Krzysztof N. Waliszewski

Instituto Tecnológico de Veracruz, Unidad de Investigación y Desarrollo de Alimentos, Veracruz, Veracruz, México

The use of cholinesterase activity as a biochemical method for monitoring organophosphate pesticide exposure in cattle is described herein. Determination of cholinesterase activity of whole blood, erythrocyte, and plasma was carried out according to the Ellman modified kinetic method. The mean baseline acetylcholinesterase activities of 9.549 ± 3.619 IU/mL in whole blood, 9.444 ± 3.006 IU/mL in erythrocytes, and 0.149 ± 0.063 IU/mL in plasma were estimated for steers from the control group. Results of multivariate analysis showed that the general responses between the control and experimental groups (in vivo, monitoring and case studies) treated with Coumaphos and Fenthion were statistically different, and the general responses of these experimental groups were statistically different over time as well. Among the fractions that were analyzed, the erythrocyte acetylcholinesterase activity could be adequate for the diagnosis of exposure or acute poisoning in cattle as it showed a good within-run and between-run precision with CVs <10% better than those in plasma.

Keywords: *Organophosphate pesticides; cholinesterase activity; cattle; whole blood*

INTRODUCTION

Biomarkers are sensitive and cost-effective tools for screening, monitoring exposure, or identifying environmental and health risks for organisms. Determination of cholinesterase activity (ChE) represents a rapid and widely used tool for detection of poisoning or exposure to antiChE compounds in mammals, birds, and fish (*1*−*3*). Many of these ChE inhibitors (organophosphates and carbamates) are being used increasingly as insecticides, and the possible adverse health effects in animals and humans are of great concern today (*4*). In Mexican tropical regions, organophosphate insecticides are widely and intensively used in agriculture and in vector control programs of Dengue fever, and for insect pest and parasite control in livestock. Most of the cattle poisoning in the state of Veracruz (Mexico) has been due to animal access to improperly disposed materials or "empty" containers, or management negligence involving the use of inadequate pesticide concentration, frequent applications, or dipping stressed cattle (*5*).

In vertebrates, two types of cholinesterases (ChE) are present: erythrocyte cholinesterase, also known as acetylcholinesterase (AchE, EC 3.1.1.7) or true cholinesterase, which is attached to erythrocytes, and hydrolyzes acetylcholine in red blood cells, myoneural junctions, cholinergic nerve ends, and the central nervous system, and plasma cholinesterase (BuChE, EC 3.1.1.8), also known as butyrylcholinesterase, which is found in plasma and different organs of the body such as liver and muscle, which hydrolyzes acetylcholine and other choline esters mainly in the plasma (*4*, *6*, *7*). Cholinesterase activity can be measured in whole blood, erythrocytes, or plasma. Use of whole blood has been recommended as a guideline for international standardization of ChE measurement (*8*). It has been suggested that whole blood ChE monitoring should be adequate for livestock species such as cattle, horses, and sheep in which ≥90% of the total cholinesterase activity is in red blood cells (*9*). Other investigators have developed a protocol for biomonitoring organophosphate nerve agent exposure in livestock. According to this protocol, exposure to ChE-inhibiting compounds would be suspected when blood ChE activity in a sentinel group is >20% below the individual baseline ChE activity (*10*). Numerous protocols are available for quantifying ChE activity; however, they have limitations. Some of these assays estimate the level of exposure to one type of ChE, require large blood samples, or are very time-consuming. Moreover, measurement of baseline blood AchE and BuChE activities in nonexposed animals is necessary before describing the effects of ChE inhibitors (*11*−*15*).

The purpose of this study was to evaluate the use of whole blood, erythrocyte, and plasma cholinesterase activity as a biochemical method for monitoring organophosphate pesticide exposure and to improve the diagnosis of organophosphate poisoning of cattle produced in tropical areas.

MATERIALS AND METHODS

Reagents. Analytical grade sodium chloride, Hepes buffer, acetylthiocholine iodide, 5,5′-dithiobis(2-nitrobenzoic acid)

* To whom correspondence should be addressed. Telephone: (5229) 34-2075. Fax: (5229) 34-4053. E-mail: vpardío@yahoo.com.mx.

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(DTNB), and iso-OMPA (tetraisopropyl pyrophosphoramid) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO) and ICN Pharmaceuticals, Inc. (Irvine, CA). Coumaphos [*O*,*O*-diethyl-*O*-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate] was obtained from Bayer de México, S.A. de C.V. Deionized water obtained by reverse osmosis (Elga Ltd., High Wycombe, Bucks, England) was used.

Materials. A centrifuge (Beckman Coulter, Inc., Fullerton, CA) was used for blood centrifugation. A diode array UV−vis spectrophotometer with a kinetic-time software and a high-performance temperature controller (Beckman Coulter, Inc.) were employed to follow the change in absorbance over time to determine cholinesterase activity.

Animals. To establish the baseline mean blood ChE activity for male cattle, blood was collected from 10 healthy male steers (*Bos taurus* × *Bos indicus*) that were 16.3 ± 2.0 months old, with a mean weight of 256.5 ± 38.0 kg during 6 months from November 1999 to April 2000. The steers were housed at the Torreón del Molino experimental farm of the Faculty of Veterinary Medicine (Veracruz, México), and none were exposed to insecticides during the previous months. The cattle were fed a basic diet consisting of a concentrate, grass hay, pasture, and tap water ad libitum as part of routine management practices used at the center and were specially managed to avoid exposure to organophosphate compounds during a period of 6 months. This group was considered a control group because no cholinesterase baseline levels for cattle reported in Mexico were found.

In Vivo Study. Ten healthy male steers (*B. taurus* × *B. indicus*) that were 16.8 ± 2.7 months old, with a mean weight of 250.8 ± 43.0 kg, were housed at the Torreón del Molino experimental farm of the Faculty of Veterinary Medicine (Veracruz, México). Animals were not exposed to insecticides during the previous months. The cattle were fed a basic diet consisting of a concentrate, grass hay, pasture, and tap water ad libitum like the previous group. The animals were sprayed with Coumaphos [*O*,*O*-diethyl-*O*-(3-chloro-4-methyl-7-coumarinyl) phophorothioate] at the recommended dose of 1 mg of active ingredient/kg of body weight once every 14 days during 6 months over the same time period as the control group. The concentration and spray procedures were the same as those commonly used for routine ectoparasite control in the area. Coumaphos was chosen for this study because it is a systemic organothionophosphate that has been routinely and extensively used in the state of Veracruz as an external antiparasite treatment mainly for control of ticks (*Boophilus* spp.) on cattle (*5*). This insecticide is transformed in vivo to coroxon, which is a potent cholinesterase inhibitor (*16*).

Monitoring Studies. Forty steers (*B. taurus* × *B. indicus*) that were 15.0 ± 2.5 months old, with a mean weight of 250.0 ± 45.0 kg, allotted in four groups of 10 animals each were monitored once a month over the course of 3 months from December 1999 to February 2000. Two groups of animals were bought from Las Choapas, Veracruz, and another two groups from Acayucan, Veracruz). The cattle were fattened in a production center facility located at Tierra Blanca, Veracruz (60 km from Veracruz City), until reaching 450−500 kg according to the management practices at the center and then slaughtered at the Federal Inspection slaughterhouse.

Case Study. Samples from a third group of animals (17 blood plasma samples from steers from Coscomatepec, Veracruz) were collected after a pesticide poisoning with an overdose of Tiguvon (20%) {Fenthion [*O*,*O*-dimethyl-*O*-(4-methylthio-*m*-tolyl) phosphorothioate]} occurred. Blood samples were collected into tubes containing no anticoagulant and delivered to the laboratory by the producer.

A preliminary study was carried out before the study started, and the levels of AchE and BuChE in whole blood fractions were determined before Coumaphos treatment started in all studies except for the case study.

Blood Sampling. Blood samples (10 mL) were obtained in the morning from the caudal vein in tubes containing K_3-EDTA as an anticoagulant. Blood samples were collected once a month at 9 a.m., and those samples from animals of in vivo studies were collected before the treatment started and then once a month 24 h after Coumaphos spraying. Samples were transported immediately to the laboratory in coolers and processed for analyses. Plasma was separated from the cells by centrifugation (3500*g* for 15 min) and removed. The erythrocytes (RBC) were washed three times with an isotonic saline solution and centrifuged three times. Samples were refrigerated (4 °C) for assays which were performed on the same day. To determine whole blood ChE activity, a 200 μL aliquot of blood was diluted in 50 mL of an isotonic saline solution (1:4), shaken, and kept at 25 °C during the assays. To determine erythrocyte ChE activity, a 200 μL aliquot of red blood cells was diluted in 50 mL of an isotonic saline solution (1:4), shaken, and kept at 25 °C during the assays. The plasma fraction was used undiluted to determine plasma ChE activity.

Spectrophotometric Assay. Determination of cholinesterase activity of whole blood, erythrocyte, and plasma was carried out according to the kinetic method of Ellman (*17*), modified by the authors by addition of 2.5 mL of Hepes buffer (pH 8.0, 0.1 M), 20 μL of acetylthiocholine iodide as a substrate (0.1 M), and 100 μL of 5,5′-dithiobis(2-nitrobenzoic acid) (0.01 M) as a chromogenic indicator to 500 μL of diluted whole blood or erythrocyte and to 500 μL of undiluted plasma. In a preliminary study, sample dilution, sample, and reagent volumes were tested in triplicate using enzyme kinetics. Absorption spectra were recorded in a diode array UV−vis spectrophotometer provided with kinetic-time software, and the temperature was kept at 25 °C using a high-performance temperature controller (Beckman Coulter, Inc.). To study the dilution influence on absorption spectra, spectrophotometric wavelength scans from 340 to 420 nm were carried out on diluted fresh samples with reagents except acetylthiocholine at the final concentration of the assays. Absorbance at 412 nm was recorded for 7 min at 25 °C, and a substrate-less blank correction was run. Cholinesterase activity was expressed as international units per milliliter (micromoles of acetylthiocholine hydrolyzed per milliliter of sample per minute) and was calculated using the formula final activity ChE (IU/mL) = $(dA/dt)F$, where (dA/dt) is the mean variation of absorbance during reading (7 min) and F is the initial dilution divided by the molar extinction coefficient.

Acetylcholinesterase levels were measured in samples before and after BuChE activity inhibition with tetraisopropyl pyrophosphoramide (iso-OMPA) selective for butyrylcholinesterase (*22*). Inhibited samples were prepared by adding 100 μL of iso-OMPA (0.004 M, Sigma), incubated with the blood fraction (500 μL of diluted whole blood or erythrocyte and 500 μL of undiluted plasma) for at least 10 min at 25 °C, before adding 20 μL of acetylthiocholine iodide as a substrate.

Repeatability. Mean between-day coefficients of variation, based on triplicate preparations from a Sera Check control bovine serum, were calculated over a period of 6 months. This control has established values for a Bayer Diagnostics cholinesterase kit[6656] of 2.5 μmol mL^{-1} min^{-1} (corrected to 25 °C) based on the Ellman reaction. To establish within-run precision, replicates of five samples of bovine whole blood, erythrocytes, and plasma were analyzed five times in 1 day.

Clinical Signs. All animals were observed for the development of clinical signs of toxicity. A clinical response was regarded as positive if an animal showed any of the typical symptoms of organophosphorus poisoning, namely, profuse salivation, ataxia, dysphonea, dullness, inappetance, diarrhea, and muscle twitching.

Statistical Analysis. ChE activities according to animal origin and time were analyzed by MANOVA analysis according to the linear model $y = (\beta_0 + \beta_1 x_1) + (\beta_2 + \beta_2 x_1)t$, using the L-Stat software run in a PowerPC Macintosh G3. The software was developed by the Chemical and Biochemical Engineering Department of Instituto Tecnológico de Veracruz and validated with Minitab version 10.5 (Minitab, Inc., State College, PA). ChE activities in blood fractions among groups of animals were compared by ANOVA followed by a Tukey test at a significance set at $P < 0.05$ using the Minitab version 10.5 statistical package.

Table 1. Means and Standard Deviations ($\bar{x} \pm$ SD) of Cholinesterase, Acetylcholinesterase, and Butyrylcholinesterase Activities in Steers from the Control Group ($n = 10$) and the in Vivo Study Group ($n = 10$) after 150 Days of Treatment with Coumaphos Once Every 14 Days[a]

	acetylcholinesterase activity (IU/mL) ($\bar{x} \pm$ SD)		butyrylcholinesterase activity (IU/mL) ($\bar{x} \pm$ SD)		cholinesterase activity (IU/mL) ($\bar{x} \pm$ SD)	
specimen	control group	in vivo group	control group	in vivo group	control group	in vivo group
whole blood	9.549 ± 3.619^a [0.67]/[0.78]	4.053 ± 1.237^b	1.201 ± 0.789^a [1.67]/[1.17]	0.571 ± 0.568^b	10.749 ± 3.783^a [0.67]/[0.81]	4.624 ± 1.354^b
erythrocyte	9.444 ± 3.006^a [0.51]/[0.56]	3.316 ± 1.381^b	4.811 ± 3.388^a [1.41]/[1.41]	3.455 ± 1.573^b	14.255 ± 4.978^a [1.29]/[0.28]	6.771 ± 2.606^b
plasma	0.149 ± 0.063^a [0.07]/[0.06]	0.066 ± 0.048^b	0.072 ± 0.052^a [5.20]/[4.96]	0.023 ± 0.014^b	0.220 ± 0.083^a [0.25]/[0.24]	0.089 ± 0.056^b

[a] Coefficients of variation (percent) (in brackets) obtained for within- and between-run precision assays in steers from the control group ($n = 10$) after 150 days. Values with different letters between columns for each enzyme activity are statistically different ($P < 0.05$).

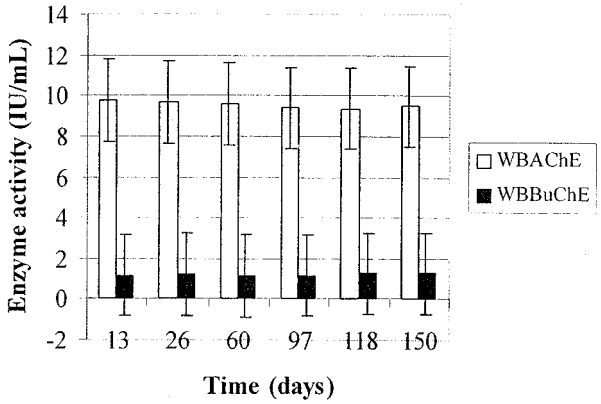

Figure 1. Variability of whole blood cholinesterase baseline activities in steers from the control group (WB, whole blood; AChE, acetylcholinesterase; and BuChE, butyrylcholinesterase).

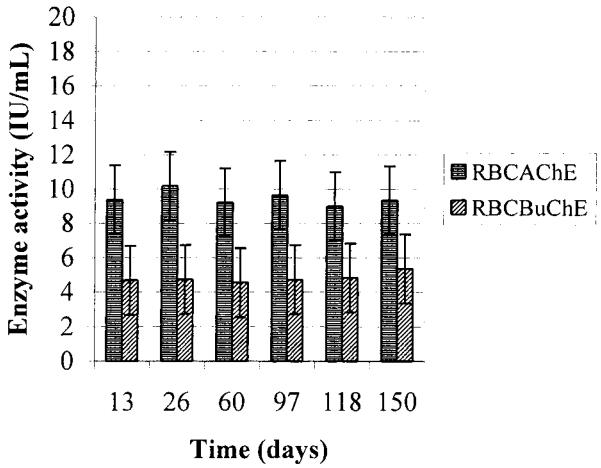

Figure 2. Variability of erythrocyte cholinesterase baseline activities in steers from the control group (RBC, red blood cells; AChE, acetylcholinesterase; and BuChE, butyrylcholinesterase).

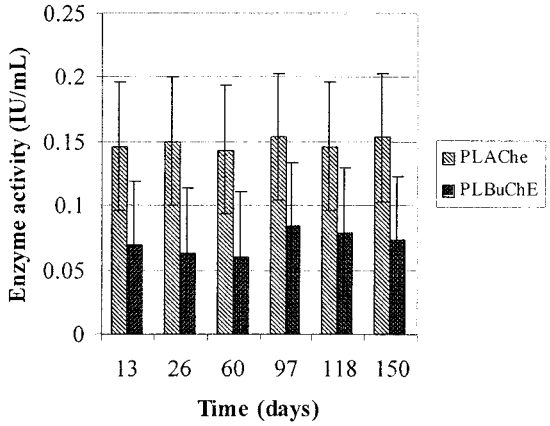

Figure 3. Variability of plasma cholinesterase baseline activities in steers from the control group (PL, plasma; AChE, acetylcholinesterase; and BuChE, butyrylcholinesterase).

RESULTS AND DISCUSSION

Mean final ChE baseline activities in steers from the control group after 5 months and results of within- and between-run precision assays are shown in Table 1. Most of the fractions that were analyzed exhibited good within-run and between-run precision with CVs of <10%, where plasma BuChE activity showed the highest CVs (5.20 and 4.96%). These CV results are comparable with those reported previously (*7*, *18*). The variation of ChE, AChE, and BuChE activities in whole blood, erythrocyte, and plasma between sampling dates for the control group was assessed by ANOVA followed by a Tukey test at a significance set at $P < 0.05$. Results indicated that time had no significant influence on ChE activities for the control group. The whole blood, erythrocyte, and plasma cholinesterase baseline activities of the control group during the study period are shown in Figures 1−3. Individual means for each activity and fraction based on six monthly samples did not differ from each final mean by more than 5%. These data indicated that a period of 6 months will be adequate to establish individual baseline ChE activities. Due to the variability of blood cholinesterase activity in healthy animals, related to the effect of intrinsic biological or environmental sources which are difficult to predict such as age, gender, breed, state of health, and climate (*9*), pre-exposure blood ChE activities must be measured from the same animal, and this mean ChE activity must be used as a baseline. This is a critical factor for biomonitoring and detecting depressions in blood ChE activity of cattle due to exposure or poisoning with cholinesterase-inhibiting pesticides.

Results show that the Ellman's acetylthiocholine hydrolysis assay can be applied to whole blood fractions for monitoring organophosphate exposure in cattle. With this methodology, the mean baseline acetylcholinesterase activities of 9.549 ± 3.619 IU/mL in whole blood, 9.444 ± 3.006 IU/mL in erythrocytes, and 0.149 ± 0.063 IU/mL in plasma were estimated for steers from the control group. ChE activities in whole blood of normal cattle using Ellman's methodology have been reported: a mean blood cholinesterase activity of 3.37 IU/mL for beef cattle (mixed breed) (*10*), a mean RBC AChE activity of 4.4 IU/mL in five crossbred Hereford steers

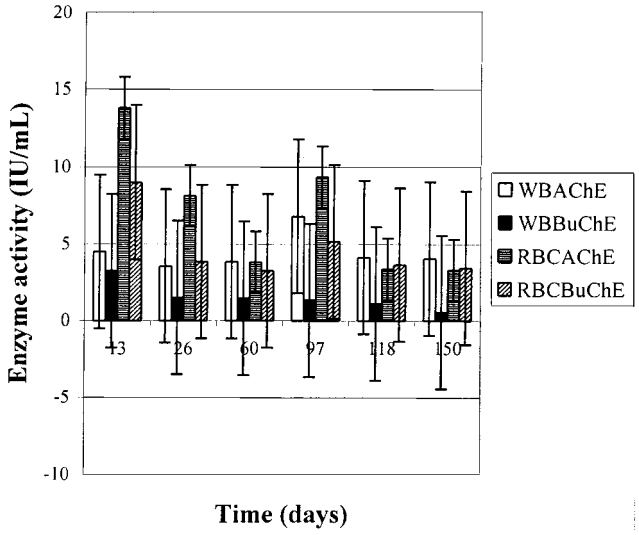

Figure 4. Effect of topical Coumaphos administration once every 14 days on whole blood and erythrocyte AChE and BuChE activities in steers from the in vivo group after 150 days of treatment (WBAChE, whole blood acetylcholinesterase; WBBuChE, whole blood butyrylcholinesterase; RBCAChE, erythrocyte acetylcholinesterase; and RBCBuChE, erythrocyte butyryl:cholinesterase).

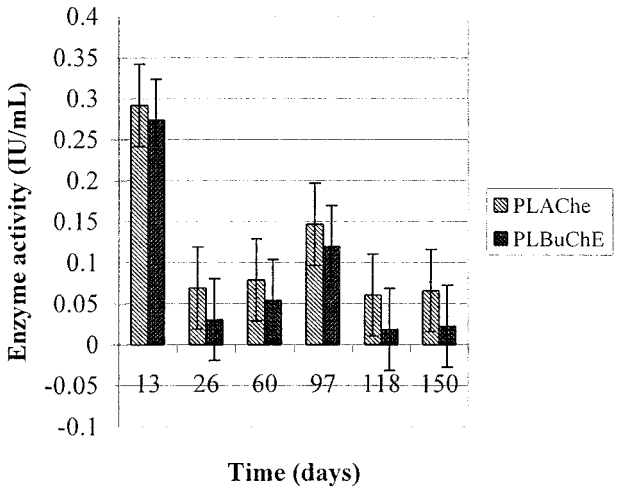

Figure 5. Effect of topical Coumaphos administration once every 14 days on plasma AChE and BuChE activities in steers from the in vivo group after 150 days of treatment (PLAChE, plasma acetylcholinesterase; and PLBuChE, plasma butyrylcholinesterase).

(*19*), and normal mean cholinesterase activity levels of 5.51 IU/mL in whole blood and 0.19 IU/mL in plasma for steers (1−2 years old) (*6*).

Acetylcholinesterase was the predominant form of cholinesterase present on the three fractions analyzed with low levels of butyrylcholinesterase in plasma. Acetylcholinesterase activity in the erythrocytes represented 98.9% and plasma 1.5% of the whole blood AChE activity. These results agreed with a previous work (*6*) which reported 95.3% of cholinesterase activity in these blood cells and a 4.7% contribution of plasma to whole blood cholinesterase activity in bullocks that were 1−2 years old. In most mammals, RBC ChE activity accounts for at least 80% of blood ChE activity (*9*, *15*). This distribution can be useful because most of the cholinesterase measurements made in animals use only acetylthicholine as a substrate (*15*, *19*, *20*), as has been recommended by the National Committee for Clinical Laboratory Standards (*21*) for acetylcholinesterase determination.

In Vivo Studies. The mean cholinesterases activities of the steers from the control group and from the animals of the in vivo study after treatment for 5 months are shown in Table 1. Results of multivariate analysis showed that the general responses were different between the control and in vivo groups ($P < 0.001$) and the responses within the in vivo group were different over time ($P < 0.005$). This means that the variables determined for the in vivo group were statistically different during the sampling period. The mean acetylcholinesterase, butyrylcholinesterase, and cholinesterase activities in the whole blood, erythrocyte, and plasma of the 10 steers from the in vivo study group were lower ($P < 0.05$) than the mean acetylcholinesterase, butyrylcholinesterase, and cholinesterase activities of the control group. Enzyme depression was observed in steers from the in vivo study 24 h after the first application of the insecticide. As shown in Figures 4 and 5, WB AChE, RBC AChE and RBC BuChE, PL AChE, and PL BuChE activity levels decreased significantly ($P < 0.05$) at day 60, recovered by day 97, and were once more depressed ($P < 0.05$) on days 118 and 150. These results agree with previous findings (*23*), in which was reported a return of whole blood acetylcholinesterase activities by day 42 to the pretreatment activities in eight Holstein-Friesian calves (3 months of age) treated with Famphur at three dosage levels, but the whole blood acetylcholinesterase activity of the third group of calves treated with 60.75 mg/kg Famphur was still depressed ($P < 0.05$) on day 49 when the experiment was terminated. Another study (*24*) reported that phosphamidon inactivated ($P < 0.01$) the erythrocyte and plasma cholinesterase activities 66 and 67%, 92 and 86%, and 98 and 89% after 12 h of 20, 40, and 80 mg/kg body weight doses of this pesticide, respectively, applied to 12 male buffalo calves (120 kg). On day 28 after insecticide administration, the erythrocyte and plasma cholinesterase activities recovered to 89−99 and 93−103% of normal in the animals that survived the 20 and 40 mg/kg body weight doses.

Following day 150 of treatment with Coumaphos, acetylcholinesterase activity decreased 64.9% in whole blood, 28.2% in erythrocytes, and 52.5% in plasma. Meanwhile, butyrylcholinesterase activity was depressed 57.6% in whole blood, 52.5% in erythrocytes, and 57.0% in plasma. Butyrylcholinesterase in erythrocytes and plasma yielded higher inhibition values probably due to its higher sensitivity to Coumaphos exposure than acetylcholinesterase. It is generally accepted that a >20% decrease in blood cholinesterase activity indicates exposure to organophosphate pesticides. Toxic effects are usually not observed until a level of cholinestersase inhibition of ≥50% is reached. Nevertheless, acetylcholinesterase activity in red blood cells can be depressed gradually to very low levels without clinical effects. A 10-fold uncertainty factor is proposed for animal RBC AchE inhibition as a default factor based on nonadversity of effect, the recognition that RBC AchE is not involved in nervous system function, and the molecular mechanism of ChE inhibition (*25*).

After 5 months of treatment, two animals from the in vivo study presented dullness and signs of diarrhea, although the steers were regularly managed with a health program to control parasite and bacterial diseases. These signs of diarrhea were reported in approximately 50% of the 20 animals treated topically with

Table 2. Means and Standard Deviations ($\bar{x} \pm$ SD) of Acetylcholinesterase and Butyrylcholinesterase Activities in Steers from the Control ($n = 10$), in Vivo ($n = 10$), and Monitoring Groups ($n = 40$) after 97 Days of Treatment with Coumaphos Once Every 14 and 21 Days, Respectively[a]

	activity (IU/mL) ($\bar{x} \pm$ SD) ($n = 10$)					
specimen	control	in vivo	Las Choapas-1	Las Choapas-2	Acayucan-1	Acayucan-2
whole blood						
acetylcholinesterase	9.549 ± 3.619[a]	3.900 ± 0.882[b]	5.672 ± 3.329[b]	6.303 ± 2.971[b]	11.870 ± 2.049[a]	12.290 ± 2.270[a]
butyrylcholinesterase	1.201 ± 0.789[a]	1.478 ± 0.891[a]	2.311 ± 1.469[a]	1.681 ± 1.015[a]	4.227 ± 2.888[a]	3.887 ± 3.186[a]
erythrocyte						
acetylcholinesterase	9.444 ± 3.006[a]	3.554 ± 1.404[b]	2.258 ± 1.402[b]	2.180 ± 0.858[b]	5.173 ± 5.338[b]	7.733 ± 0.785[b]
butyrylcholinesterase	4.811 ± 3.388[a]	3.676 ± 2.678[a]	1.024 ± 1.117[b]	1.313 ± 0.867[b]	1.302 ± 0.308[b]	1.218 ± 0.486[b]
plasma						
acetylcholinesterase	0.149 ± 0.063[a]	0.081 ± 0.035[a]	0.053 ± 0.043[a]	0.143 ± 0.086[a]	0.231 ± 0.322[a]	0.097 ± 0.056[a]
butyrylcholinesterase	0.072 ± 0.052[a]	0.015 ± 0.008[b]	0.055 ± 0.066[a]	0.079 ± 0.086[a]	0.029 ± 0.025[a]	0.047 ± 0.041[a]

[a] Values with different letters among columns are statistically different ($P < 0.05$).

20.25, 40.5, and 60.75 mg/kg Famphur (*23*). A blood cholinesterase inhibition level of up to 64% was observed without clinical effect in 229 cattle treated with Crufomate (70−300 mg of active ingredient/kg of body weight) (*11*). Although there was evidence of a significant decrease in whole blood, erythrocyte, and plasma cholinesterase activities of in vivo study animals, no other clinical signs of toxicity were observed. Thus, a tolerance to the organophosphate pesticide might have developed. This tolerance phenomenon has been reported and termed compensatory changes in postsynaptic cholinergic receptors (downregulation of muscarinic cholinergic receptors) and is thought to constitute a primary mechanism of tolerance to AchE inhibition (*9*, *26*).

Monitoring Studies. The mean cholinesterases activities of the steers from the control group and from the animals of the monitoring study after 3 months of treatment are shown in Table 2. Results of multivariate analysis showed that the general responses between the control and monitoring groups were different ($P < 0.001$) and the responses within the monitoring groups were different over time ($P < 0.005$). Among the enzyme activities that were analyzed, whole blood AChE activity levels of both animal groups from Acayucan were not statistically different ($P < 0.05$) from the control group despite pesticide treatment, but their levels were significantly higher ($P < 0.05$) than those of the steers from Las Choapas with levels significantly lower ($P < 0.05$) than that of the control group. Butyrylcholinesterase activity levels were not statistically different ($P < 0.05$) among monitoring groups and the control group. RBC AChE and BuChE activity levels from all monitoring groups were significantly lower ($P < 0.05$) than that of the control group. The final depression of erythrocyte AChE activity after the treatment with Coumaphos of groups of steers from Las Choapas and Acayucan seems to depend on the precedence of the animal, probably due to previous exposure to organophosphate agents of the group from Las Choapas (Figure 6). Whole blood AChE activity levels of in vivo study steers were significantly lower ($P < 0.05$) than those of the other groups, probably because Coumaphos was applied to these animals more frequently (once every 14 days). Erythrocyte AChE activity levels of in vivo study steers were significantly lower ($P < 0.05$) than the levels of the Acayucan-1 group, but were not significantly different ($P < 0.05$) from the levels of the other groups.

Case Study. Results from ANOVA analysis showed that only plasma AchE activitiy levels of the animals poisoned with an overdose of Fenthion were statistically different ($P < 0.05$) from that of the control group as shown in Table 3. Plasma AChE activity was inhibited

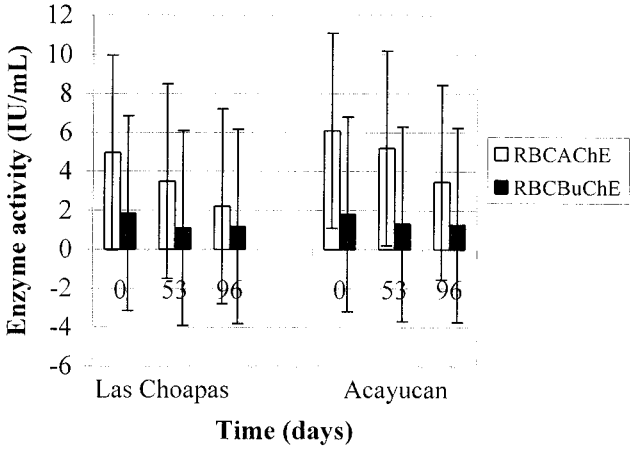

Figure 6. Effect of topical Coumaphos administration once every 21 days on erythrocyte AChE and BuChE activities in steers from monitoring groups after 96 days of treatment (WBAChE, whole blood acetylcholinesterase; WBBuChE, whole blood butyrylcholinesterase; RBCAChE, erythrocyte acetylcholinesterase; and RBCBuChE, erythrocyte butyrylcholinesterase).

Table 3. Means and Standard Deviations ($\bar{x} \pm$ SD) of Plasma Acetylcholinesterase and Butyrylcholinesterase Activities in Steers from the Control Group ($n = 10$) and the Case Study Group ($n = 17$) after Poisoning with Fenthion[a]

group	acetylcholinesterase activity (IU/mL) ($\bar{x} \pm$ SD)	butyrylcholinesterase activity (IU/mL) ($\bar{x} \pm$ SD)
control	0.149 ± 0.063[a]	0.072 ± 0.052[a]
case study	0.038 ± 0.038[b]	0.087 ± 0.089[a]

[a] Values with different letters between rows for each enzyme activity are statistically different ($P < 0.05$).

88.6% when compared with the AChE activity of the control group. All animals showed the clinical symptoms of organophosphate poisoning: ataxia, dysponea, diarrhea, and muscle twitching. Even though these animals were treated with Atropine, six of them died.

These findings reflect the fact that consecutive insecticide treatments had additive effects. Results indicate that administering Coumaphos topically at 1 mg/kg of body weight once every 14 and 21 days to cattle under normal management practices in tropical areas produces a significant inhibition in whole blood, erythrocytes, and plasma acetylcholinesterase and butyrylcholinesterase activities when compared to baseline levels. This inhibition could be influenced by the age of the animals and environmental factors.

The results of this study show that Ellman's assay could be used to confirm decreases in AChE activity in

whole blood and erythrocytes associated with organophosphate exposure. These results agree with previous works (*9*, *10*). The inhibition of erythrocyte AChE activity using Ellman's assay could be adequate for assessing exposure of cattle in risk assessment to cholinesterase-inhibiting pesticides or acute poisoning in cattle, because it gives reproducible values with better CVs than those in plasma, and 90% of the cholinesterase activity is RBC AChE. Moreover, the erythrocyte AChE is considered toxicologically relevant because of its biochemical analogy with nervous system AChE. This is consistent with the recommendations made by the World Health Organization (*27*) in human studies.

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