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EXCRETION OF METHOXYCHLOR IN COW MILK FOLLOWING DERMAL APPLICATION

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SKAARE, J. U., G. BERGE, S. ØDEGAARD and K. GRAVE: *Excretion of methoxychlor in cow milk following dermal application.* Acta vet. scand. 1982, *23*, 16—23. — The concentrations of methoxychlor in cow milk and plasma were determined following a cutaneous application of a non-commercial emulsion and an intravenous injection of a solution containing 5 and 1 g methoxychlor, respectively. Furthermore, some pharmacokinetic parameters were estimated and the results were evaluated toxicologically by comparison with the acceptable daily intake.

Maximum concentration of methoxychlor in milk, found 2 days after dermal administration, was 0.052 ppm, decreasing to around 0.005 ppm 30 days later.

From the results, a distribution volume greater than 200 times the body volume, a dermal absorption of around 1/5 of the topically applicated dose, and a half-life of 8—10 days were estimated.

It was concluded that the levels in cow milk, following dermal application with an emulsion of methoxychlor did not represent any threat to human health and methoxychlor was recommended as a pesticide of choice for use on dairy cows.

methoxychlor; cow; milk; excretion; cutaneous application.

Since the 1950's, methoxychlor (1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane) has been applied topically to dairy cows for the effective control of certain ectoparasites like hornflies, stableflies and lice (*Hoffman* 1954, *Cheng et al.* 1958). During these years several investigations on the excretion of methoxychlor in milk following dermal application have been reported (*Carter et al.* 1949, *Birk & Dixon* 1951, *Claborn & Wells* 1952, *Helrich et al.* 1958, *Cluett et al.* 1960, *DeFoliart & Willett* 1961,

Matthysse & Lisk 1963, *Hurwood* 1968, *Oehler et al.* 1969). The results from these studies are somewhat difficult to interpret since different analytical methods with varying degree of sensitivity and specificity have been used. Also, differences in dosage, formulation and application form have resulted in conflicting results which clearly demonstrate the need for further pharmacokinetic work.

In Norway, there is an increasing problem with bovine ectoparasites (Linognathus vituli and Damalinia bovis). Among the many pestcides available, none fully satisfy the requirements because of the excretion in milk.

Therefore, the aim of the present investigation was to study the excretion of methoxychlor in milk and to estimate some pharmacokinetic parameters following topical application of a non-commercial methoxychlor emulsion. A sensitive and specific analytical method was used.

MATERIALS AND METHODS

Chemicals and formulations

N-hexane and acetone were redestilled in all-glass apparatus and tested by gas liquid chromatography (g.l.c.) after 50-fold concentration. The standards, p,p-DDT (1,1,1-trichloro-2,2-bis (p-chlorophenylethane) and methoxychlor 90.0 % (1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane) (National Physical Laboratory), were dissolved in n-hexane. Aluminium oxide, Al_2O_3, neutral, 70—230 mesh (Merck) was activated at 800°C over night and deactivated with 5 % water. Tween 80, Span 60, propyleneglycol and ethanol were obtained from Norsk Medicinal Depot and A/S Vinmonopolet and were all products for pharmaceutical use. Two methoxychlor formulations, an emulsion (5 g methoxychlor, 18 g Tween 80 and 2 g Span 60 and propylene-glycol ad 100 ml — this preparation was diluted with water to 1000 ml just before use) and a solution (0.5 g methoxychlor, 10 g ethanol and propylene-glycol ad inj. ad 100 ml) were used for topical and intravenous administration, respectively, of the cows.

Animal experiments

Six stanchioned dairy cows of the Norwegian Red Cattle (NRF) breed, age 8—10 years and weighing ca 500 kg, were used. The daily rations of feed were 5 kg silage and 7.5 kg hay.

The cows were milked twice daily and the average milk production was 4439 kg per year and 9.2 kg per day during the experimental period. Butterfat content of composite milk samples averaged 4.35 %.

To 4 cows (No. 1—4) 1 liter of the 0.5 % methoxychlor emulsion was applied topically by use of a sponge. This volume was sufficient for thorough wetting of the cow excluding udders and underlines. Two cows (No. 5 and 6) received an intravenous injection of 200 ml 0.5 % methoxychlor solution. Inappentence and hematuria were observed in 2 days after injection. Cow No. 6 was not lactating. 50 ml milk samples were collected by hand milking before the start of the machine milking. To avoid contamination of the milk the udders were thoroughly cleaned prior to each milking. Due to the variations in butterfat concentration in milk during the milking process and during the day, milk samples were collected a.m. and p.m. 1,2,3,4,5,7,9,11,14,18,25 and 29 days post-treatment. Some samples were also collected following machine milking.

Blood samples were drawn in heparinized vacutainers (20 ml). During the first week post-treatment blood samples were collected at the same time intervals as milk samples. However, during the last 3 weeks of the experimental period blood samples were taken a.m. only. From cows No. 5 and 6 the first blood samples were obtained 2.5 h after the intravenous injection. The milk and plasma samples were stored at —20° until analyzed.

Determination of methoxychlor residues

The milk samples were analyzed according to the described method which was partly based on a method by *Brevik* (1978). From thoroughly mixed milk 10 ml were transferred to 100 ml test tubes and 50 ng internal standard, p,p-DDT (100 µl of 50 µg p,p'-DDT/100 ml n-hexane), 20 ml n-hexane and 15 ml acetone were added. The tubes were placed in beakers filled with ice. Following homogenization for 1 min using an ultrasonic disintegrator (70 watt Sonifier B-12, Branson Sonic Power Co), the samples were kept at room temperature for about 1 h. After centrifugation for 5 min, 3000 rpm (Roto Silenta III, Hettich), the supernatant was transferred to 100 ml test tubes. The extraction was repeated and the total n-hexane extracts were concentrated to 2—3 ml at room temperature under an air stream. The solution was transferred to calibrated 15 ml test tubes and evaporated

to dryness for determination of extractable butter fat. Separation of methoxychlor from milk fat was done by column chromatography using glass columns (0.8 mm i.d., 25 cm), filled with 8 g Al_2O_3.Following washing of the column with ca 10 ml n-hexane, 0.1 g of milk fat was applied on the column. Methoxychlor was eluted with 100 ml n-hexane. The eluates were evaporated overnight at room temperature under an air stream. Final volumes were 0.3—1.0 ml.

To 2 ml plasma samples were added 12.5 ng internal standard and 10 ml n-hexane. A whirlmixer was used for thorough mixing. The samples were left in glass-stoppered glass tubes for about 1 h. Following centrifugation at 3000 rpm for 5 min, the n-hexane phase was collected. The procedure was repeated and the total n-hexane phase was evaporated to dryness and 0.3 ml n-hexane added.

Aliquots of the n-hexane extracts were analyzed by g.l.c. using a Varian gas chromatograph Model 3700, equipped with an electron capture detector (^{63}Ni source) and a glass column (1.5 m long, 0.2 mm i.d.) filled with 4 % SF-96 on chromosorb w 100—120 mesh. G.l.c. operating conditions were as follows. Temperature: column 210°C, injector 250°C, detector 300°C; N_2 flow rate: 35 ml/min. Methoxychlor in milk and plasma samples were determined by use of a standard curve which had been prepared by analysis of blank milk samples containing internal standard and various concentrations of methoxychlor. The recoveries were 70—75 % on the 0.005 ppm and 0.05 ppm levels for milk and plasma, respectively. Linearity was observed from 0.005—0.1 ppm for milk analysis. For plasma the quantitation limit was 0.009 ppm methoxychlor.

RESULTS

The excretion of mehoxychlor in cow milk is illustrated in Fig. 1. As can be seen, the dermal application of 5 g methoxychlor in an emulsion resulted in a maximum level in milk of 0.052 ppm 2 days post-treatment. Following intravenous administration of 1 g methoxychlor the level, being only 0.012 ppm at the first milking 4 h after injection, reached a maximum of 0.114 ppm at the next milking 14 h later. Throughout the experimental period the 2 different modes of application resulted in similar methoxychlor levels in milk, and 29 days post-treatment levels were around 0.005 ppm (Fig. 1).

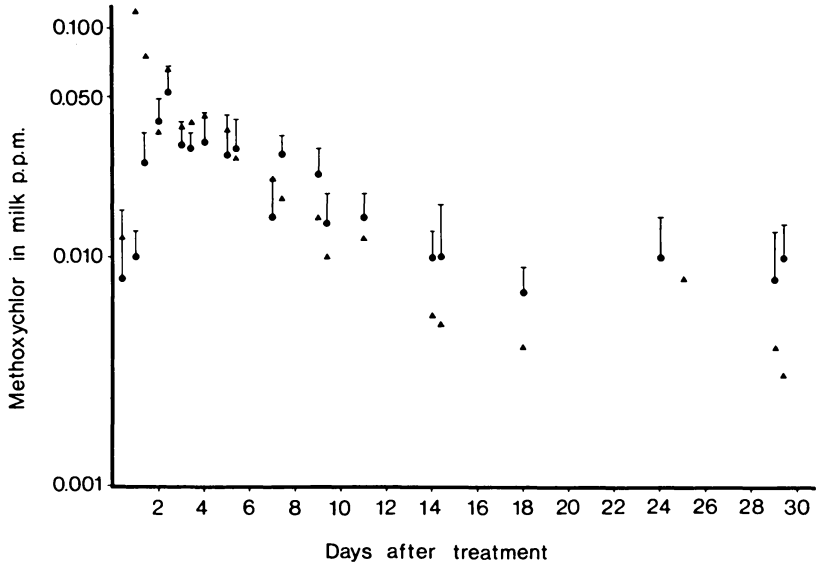

Figure 1. Excretion of methoxychlor in milk in 4 cows washed with an emulsion containing 5 g methoxychlor (●) and in 1 cow given 1 g methoxychlor intravenously (▲). Mean + s are given.

The concentration of methoxychlor in plasma was 0.009 ppm 2.5 h following intravenous injection of 1 g. At subsequent samplings the levels were below 0.009 ppm which was the lower limit of quantitation for plasma. Furthermore, methoxychlor residues were detected in blood samples from 1 of the cows which had received the topical application. From the remaining cows no blood samples were analyzed.

DISCUSSION

The methoxychlor levels in plasma after dermal application were all below the quantitation limit of the analytical method used. However, a plasma level of 0.009 ppm methoxychlor shortly after the intravenous injection and certainly before the establishment of distribution equilibrium indicated a distribution volume greater than 200 times the body volume. Because of the high lipid solubility of methoxychlor a large distribution volume would be expected.

During the experimental period all the cows were low yielding. However, milk yields did not differ significantly between cows. Thus, the finding that levels of methoxychlor in milk were

similar following intravenous and cutaneous application of 1 and 5 g of the pesticide, respectively, indicated that the systemic availability was around 1/5 of the dermally applicated dose. Furthermore, a large distribution volume of a compound is often resulting in a long half-life, and for a lipid soluble substance like methoxychlor significant elimination in milk is expected to occur. However, only a few mg (< 5 mg) was excreted in milk during the first month after intravenous injection of 1 g methoxychlor. It can therefore be assumed that significant storage in body compartments was occurring, resulting in a long half-life and a slow excretion of methoxychlor in milk. Using the excretion curve (Fig. 1) a half-life of 8—10 days may be postulated. This corresponds well with the results of *Reynolds et al.* (1976) who estimated the half-life to be 10 days from the depletion of methoxychlor from the bodyfat of sheep.

From a therapeutical point of view, application of a suspension or an emulsion in water would be better than dusting. The former, however, is expected to result in higher dermal absorption. Dusting with 100 g of a 5 % powder gave rise to a maximum concentration of 0.6 ppm in milk fat (approximately 0.024 ppm in whole milk) (*Bjerk & Westbye*, unpublished) compared to 0.073 ppm following application of the same amount of methoxychlor in an emulsion in the present study. Higher residues in milk were also found after spraying than after dusting by *Cluett et al.* (1960).

The results of earlier investigations seem somewhat conflicting. The highest residues reported, 0.7 ppm (*Claborn & Wells* 1952) and 0.46 ppm (*Hurwood* 1968) found after application of an emulsion, are clearly higher than the findings reported here. Application of suspensions has resulted in methoxychlor levels in milk of 0.4 ppm (*Claborn & Wells*), 0.05—0.06 ppm (*Birk & Dixon* 1951) and 0.15 ppm following a large-volume spray (3 gal of 0.2 % methoxychlor) (*Matthysse & Lisk* 1963). Even lower methoxychlor levels have resulted from dusting (*Cluett et al., Matthysse & Lisk*). Thus, the degree of absorption and the residue level seem to depend partly on the formulation used.

Finally, toxicological evaluation of the methoxychlor residues found in milk after ectoparasite treatment was attempted. In comparison with the acceptable daily intake (ADI-value) of 0.1 mg/kg, proposed by FAO/WHO (1978), the residue values found by us would correspond to a daily milk consumption of about

1 l/kg (body weight). In Norway, for the control of ectoparasites, 2 treatments will be undertaken 10—12 days apart only once or twice yearly in a limited number of herds. Thus, methoxychlor can be recommended as a pesticide of choice for use on dairy cows.

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SAMMENDRAG

Utskillelse av metoksyklor i kumelk etter dermal applikasjon.

Metoksyklor-nivå i kumelk og plasma ble bestemt etter dermal applikasjon av en ikke-kommersiell emulsjon og en intravenøs injeksjon av en løsning inneholdende henholdsvis 5 og 1 g metoksyklor. Videre ble noen farmakokinetiske parametre forsøkt bestemt og resultatene ble evaluert toksikologisk ved sammenligning med det akseptable daglige inntak (ADI-verdi) (WHO/FAO 1978).

Maksimumsverdier av metoksyklor i melk var 0.052 ppm 2 dager etter dermal applikasjon. Nivået var ca 0.005 ppm etter 30 dager.

Distribusjonsvolumet ble estimert større enn 200 ganger kroppsvolumet, den dermale absorpsjon ca 1/5 av applisert dose og halveringstiden 8—10 dager.

Det ble konkludert med at nivåene i kumelk etter dermal applikasjon av en metoksyklor emulsjon ikke representerer noen helserisiko og metoksyklor kan anbefales som et pesticid til bruk på melkekuer.

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