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Chemical substances and chemical preparations

Release of propoxur from collars

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Data Sheet

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Summary

Propoxur is the active ingredient in collars used against fleas on dogs and cats, and the collars are claimed to effectively protect the animal for a period of 3 - 5 months due to a continuous release of propoxur during the period of use.
In the present study rate of release of propoxur from collars has been examined during a period of 3 months in order to find out, whether rate of release is constant or changing during the period of use. Two dogs have been wearing a modified collar during the period and at different times samples from the collars have been analysed for remaining content of propoxur.
It was found that rate of release of propoxur was highest immediately after application of the collar and gradually decreased during the three months period. No significant difference was observed between the two collars examined as well as no significant difference was observed between collars applied on two different dogs.
Furthermore, it was concluded that physical stress (bending, twisting etc.) to the collar may contribute significantly to the release of propoxur, especially in the beginning of the period of use.

1 Introduction

Propoxur (figure 1) is a carbamate insecticide, which is used world-wide against several types of pests (ants, cockroaches, bugs etc.) in households. In Denmark propoxur is only registrered for use against fleas on dogs and cats. Different formulations are on the market, but impregnated collars are by far the most dominant formulation type (Miljøstyrelsen, 1993). Collars impregnated with propoxur are marketed for use on either cats or dogs; the difference being the size of the collar and not the composition of the material. Collars impregnated with propoxur are the only impregnated collars on the Danish market, but collars from many different companies are registrered for use. In 1993 23 different products (12 collars for cats and 11 collars for dogs) were on the market (Miljøstyrelsen, 1993). The size and shape of the collars differ slightly from product to product, but all collars on the Danish market are made of polyvinyl chloride and contains *ca.* 10 % propoxur as the active ingredient.
Collars are claimed effectively to protect the animal from infestation of fleas for 3 - 5 months, and the long period of insecticidal activity relies on a continuous release of propoxur from the collar. During the last 5 years several cases have occurred, where cats have become sick and have shown signs of intoxication when wearing propoxur-containing collars (Miljøstyrelsen, personal information). In a typical situation the cat has become sick shortly after application of the collar, and the question has arised whether release of active ingredient occurs uniformly over the claimed period of effectiveness, or a high amount of propoxur is released immediately after or during application of the collar.
The purpose of this investigation was to demonstrate the time profile of the release of propoxur from collars under realistic conditions in order to help answering this question.

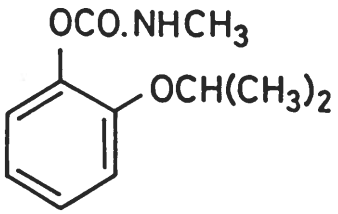

Figure 1. Chemical structure of propoxur.

2 Experimental

2.1 Design of experiment

To achieve results showing a realistic time profile of the release of propoxur from collars, it was desided to apply collars on two dogs kept as pets by collegues at The National Environmental Research Institute (NERI). In spite of the lack of possibility to control various conditions (temperature, air humidity, air flow etc.) as in a laboratory experiment, an experiment involving animals was chosen, because it reflects more closely realistic conditions. At certain intervals samples from the collars were taken and the residual content of propoxur was measured. In this way release of propoxur was expressed as a decrease in remaining content of propoxur in the collar.

2.2 Materials and methods

Dogs. Two dogs; one labrador retriever, 8 years old, weight 38 kg, height 52 cm, (dog no. 1) and one crossbreed (half labrador retriever and half collie), 6 years old, weight 30 kg, height 40 cm, (dog no. 2).

Collars. Samples of the product "Matas Loppehalsbånd til hunde" commercially available on the Danish market; a collar registrered for use against fleas on dogs. Declared content: 2.9 g propoxur/-collar (~ a concentration of 9.8 %). Length of collar: 65 cm.

Chemicals. Propoxur PESTANAL® analytical standard, acetonitrile (HPLC grade).

Preparation of collars. All handling of collars during preparation was performed as gentle as possible to reduce release of propoxur from the collars. Immediately after opening of the packages 18 pieces of approximately equal size were cut from the uniformly shaped central part of each of two collars (figure 2a). Two holes (3 mm) were drilled in each piece of collar, and the pieces were assembled using ordinary nylon cable ties (Tyraps®) to form two double-stranded collars. Each strand consisted of nine pieces cut from the same initial collar, but the two strands consisted of pieces from different collars. The two buckles and the opposite ends from the initial collars were fixed to the double-strands again using nylon cable ties. A drawing of a final collar used in the experiment is shown in figure 2b. Finally the collars were applied on the two dogs.

Sampling of pieces of collar. At the time of sampling a piece was cut loose from the collar and immediately after transferred to a glass vial, which was sealed air-tightly. A pair of pieces (one from each strand) were taken at every time of sampling. The loose ends of the collar remaining on the dog were connected using an additional nylon cable tie. Samples were analysed on the same day as they were taken from the collar.

Determination of propoxur content. Content of propoxur in pieces of collar was determined using HPLC following Soxhlet extraction of active ingredient from the collar matrix. Details of the analytical method is described in appendix I.

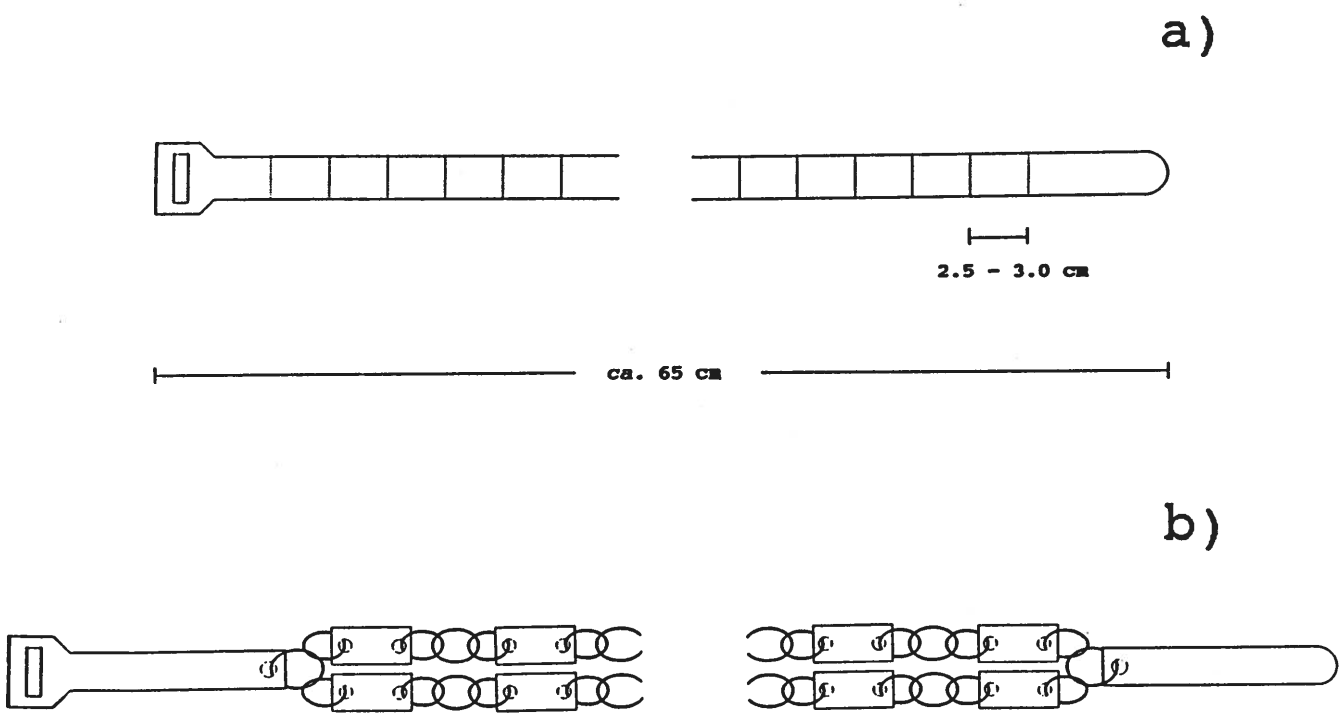

Figure 2. Outline of collar before and after preparation for the experiment. a) original collar, b) modified collar as used.

3 Results and discussion

The basic reason for choosing an experimental design where collars are applied on living animals in stead of conducting a laboratory experiment was the possibility to create realistic conditions for release of active ingredient from the collar. Although the concern about rate of release of propoxur stemmed from cases involving cats, dogs were chosen as animal for wearing the collar in order to diminish the risk of the animal getting sick during the experimental period. In order to be able to evaluate whether differences in external conditions had any influence on the release of propoxur, two dogs living at different places were included in the study. Furthermore, to be able to evaluate whether release of propoxur differed from collar to collar, two different collars where represented on each dog.
Initially several parameters had to be examined, before we decided choosing an experimental design, which involved cutting the collar into pieces and indirectly measuring release of propoxur by measuring the residual amount in the collar: a) the method of analysis, b) the homogeneity of a collar in its longitudinal direction and c) the loss of propoxur during preparation of collars.

3.1 Validation of the method of analysis

The method used for determination of content of propoxur in pieces of collar was essentially a method (Slahck, 1984) approved as official method by the two organisations Association of Official Analytical Chemists (AOAC) and Collaborative International Pesticides Analytical Council (CIPAC). The method is approved for analytical chemical control in order to establish whether formulated products comply with specification. However, in order to demonstrate its applicability in the present study, additional validation with respect to precision and detectability was necessary. The reason for this was the smaller amount of sample available for analysis compared to what is described in the official method. This could have an effect on method precision. A determination of detection limit of the method was also considered necessary to make sure that the method would be able to satisfactory analyse pieces of collar with low residual content of propoxur. Validation of the analytical method is described in detail in Appendix I. Results showed a precision of 0.8 % and a detection limit of 0.23 mg propoxur/piece of collar (corresponding to *ca.* 0.15 % of initial content), which were considered satisfactory.

3.2 Homogeneity of collars

In this study release of propoxur was determined as the difference between initial content and residual content at the time where a piece of collar was removed from the dog. An investigation of homogeneity with respect to distribution of propoxur in longitudinal direction of a collar was necessary, to make sure that the content found in one piece of collar taken at the beginning of the time course, would represent the initial content of all the other pieces derived from the same collar. In table 1 results are shown from an experiment, where pieces of collar taken at four different places along the collar were analysed. The results showed no significant difference between individual contents, which indicated a homogeneous distribution of propoxur in longitudinal direction of the collar.

Table 1. Distribution of propoxur in longitudinal direction of collar (length, 65 cm).

Sample no.	Distance from buckle, cm	Content of propoxur[a)]
1	2 - 5	9.8 ± 0.2
2	23 - 26	9.7 ± 0.2
3	39 - 42	9.8 ± 0.2
4	55 - 58	9.9 ± 0.2

a) Single determination ± 95 % confidence limits.

3.3 Loss of propoxur during preparation of the collar

From the beginning of this study it was clear, that all handling of and manipulation with collars caused some release of propoxur from the collar matrix. Even very gentle handling or bending of the collar made the surface look white (in contrast to the initial light brown colour) due to fine powder being released. It was therefore obvious that it was impossible to avoid some release of propoxur during the various steps of preparation (cutting, drilling of holes and reassembling).

The amount of propoxur lost during preparation of the collar and when pieces of the final collar were taken for analysis was examined by a) comparing contents found before and after preparation and b) comparing contents found following repeated sampling from the same collar. Results from that experiment (performed twice) are shown in table 2.

Results from analysing a collar before and after lying for two hours on a table are also included in the table, to demonstrate that the measured loss of propoxur was not a result of a free evaporation taking place during the first hours after opening of the package.

The results from four repetitive samplings of pieces of collar showed no apparent influence of sampling on the content of propoxur in the remaining pieces of collar. A decreasing content in samplings 1 - 4 would have been expected, if the sampling itself gave rise to release of propoxur. Since this was not found, it was concluded that the sampling procedure did not cause any release of propoxur. In that case the results from the four repititive samplings reflects variations in loss of propoxur from pieces of collar during preparation, and a difference between the mean value and initial content reflects loss of propoxur during preparation. Calculated in this way the loss of propoxur (expressed as % loss) is included in the table too. The results showed that a significant amount (6 - 7 % of initial content) of propoxur was lost during preparation of the collar, but variation of the lost amount was reasonably low. This was essential to be able to maintain the assumption that the content found in one piece of collar taken at the beginning of the experimental period, would represent the initial content of all the other pieces derived from the same collar.

By comparison with a collar left on a table for two hours it was clearly demonstrated, that the loss of propoxur actually was a result of physical stress of the collar during preparation and not due to free evaporation. Nevertheless, from this experiment it was clear that the significant loss of propoxur during preparation had to be taken into account when samples for determination of "initial content" (t = 0, in relation to time of use of collar) were taken. As a consequence it was decided that these samples had to be taken immediately after preparation of the collars.

Table 2. Amount of propoxur lost from collars during preparation and sampling of pieces of collar.

Content of propoxur[a], %						Loss of propoxur, %
Initial	After preparation[b]				Without preparation	
	1	2	3	4		
10.2 ± 0.2	9.1 ± 0.3	9.4 ± 0.3	9.6 ± 0.3	9.8 ± 0.3	-	7.2
9.9 ± 0.2	9.0 ± 0.3	9.4 ± 0.3	9.3 ± 0.3	9.1 ± 0.3	-	6.6
10.0 ± 0.2	-	-	-	-	10.1 ± 0.2	-0.6

a) Mean of two determinations ± 95 % confidence limits.
b) Following preparation and application of the collar a sampling situation was simulated. Four repetitive samplings were performed.
c) Following lying on a table for two hours.

3.4 Release of propoxur from collars

Results from determination of remaining content of propoxur in the pieces of collar sampled during the experiment are shown in table 3. Unfortunately one of the two dogs (dog no. 1) had lost its collar on day 45 of the experiment, which explains why results from the two last samplings from dog no. 1 are missing. Graphical presentation of the results (expressed as % of initial content remaining in the collar) are shown in figure 3. Time courses for the two different batches of collar applied on each dog are shown individually. Fluctuations are appearing in the beginning of the time courses, but the general trend of all four graphs is the same: Release of propoxur being high at the beginning, gradually decreasing, and being very low at the end of the time course. It is also characteristic that 40 - 50 % of the initial content is remaining in the collar at the end of the experiment (~ end of recommended period of use).
Fluctuations appearing in the beginning of the time courses are very likely caused by variations in handling the pieces of collar during sampling and are not considered to be variations in properties of the collar. It is apparent from working with these collars, that propoxur is released to the surface of the materiel by physical handling (bending, twisting etc.), and variations are impossible to avoid when sampling from an animal. The fact that variations are relatively highest in the beginning of the time course agrees with this assumption. Propoxur is released from the

Table 3. Results from determination of remaining content of propoxur in pieces of collar during the experimental period.

Day no.	Content of propoxur, %[a]			
	Dog no.1		Dog no. 2	
	Collar no. 1	Collar no. 2	Collar no. 1	Collar no. 2
0	8.23	9.10	7.91	8.76
1	8.01	8.22	8.47	7.95
2	6.80	6.93	8.14	6.68
4	5.26	6.27	7.42	6.84
7	6.33	5.65	6.27	7.41
14	5.01	4.86	5.56	6.28
28	4.74	4.70	5.23	4.73
56	nd[b]	nd[b]	5.42	4.92
105	nd[b]	nd[b]	4.35	4.50

a) Single measurements.
b) Not determined (see text).

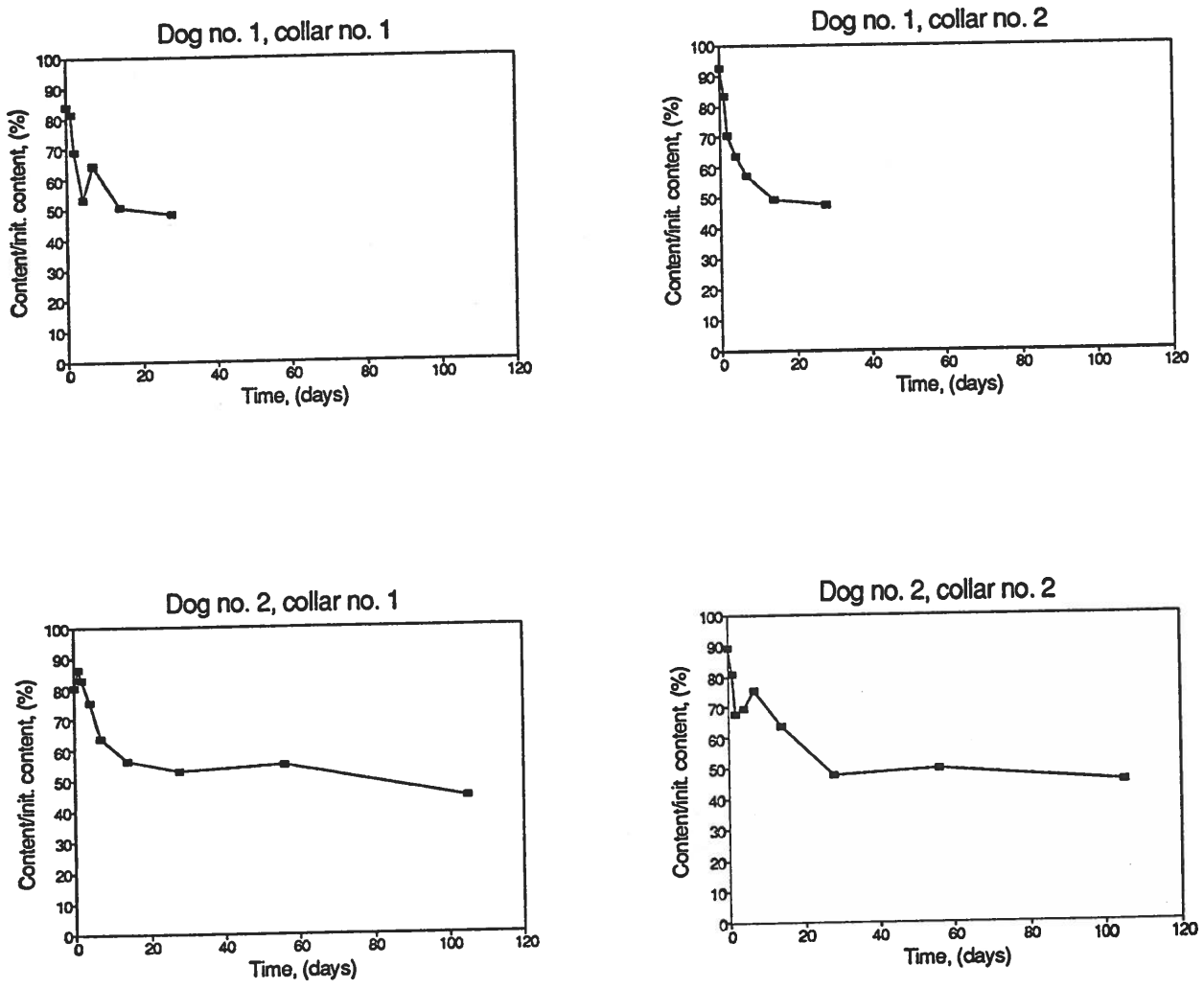

Figure 3. Time courses showing amount of propoxur remaining in collar during the experimental period. Individual results.

surface, and a relatively high proportion of the propoxur remaining in the collar towards the end of the experiment is expected to be distributed in the core of the material. It will therefore be less influenced by variations in physical handling during sampling.

Statistical treatment of the results showed no significant differences between the collars involved in the investigation. Furthermore no significant difference was found between results obtained from the same collar, but worn by different dogs. An illustration of this is shown in figure 4; a graphical presentation of mean values of parallel results from either same collar or same dog. Only part of the time course from which all results were available is shown.

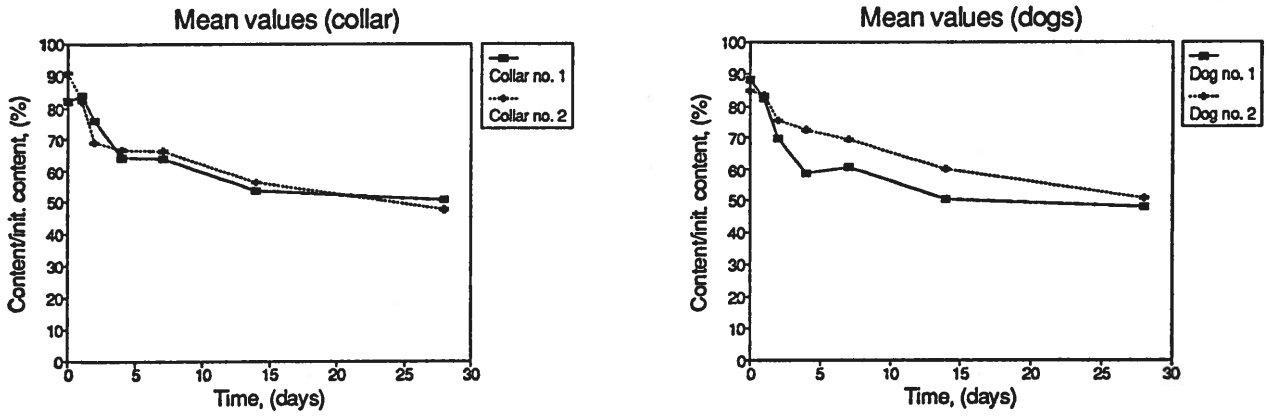

Figure 4. Time courses showing amount of propoxur remaining in collar during the experimental period. Mean results (of collars and of dogs).

Finally a time course showing overall mean values (eliminating collar differences as well as external factors) is given in figure 5. Standard deviations, which are also included on the graph, illustrate to some extent the higher variation in the beginning of the experiment resulting from handling during sampling.

Generelly the smooth curve indicates a gradually decreasing rate of release of propoxur from the collar. There is no indication for an exceptionally high rate of release just after the collar has been put into use. By comparing results from the time course study with observations during initial experiments (loss of propoxur during preparation of collars and no loss, when a collar was left lying on a table) two possible explanations can be given for cases where cats have been intoxicated by propoxur collars. One possible explanation is, that cats in some cases become intoxicated by propoxur released "naturally" as a result of inherent properties of the collar formulation. Rate of release is highest in the beginning of the period of use of the collar and this could perhaps in some cases lead to levels exceeding lower limits for developing symptoms of intoxication.

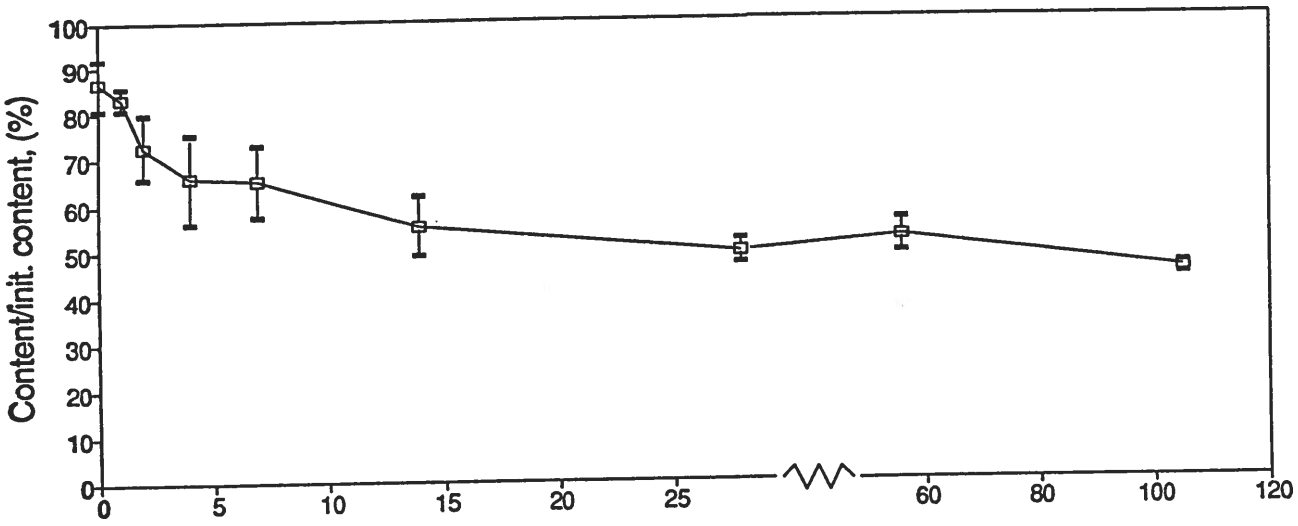

Figure 5. Time course showing amount of propoxur remaining in collar during the experimental period. Overall mean results. Standard deviations are indicated by vertical bars (n = 4, except for results on days 56 and 105 where n = 2).

Another explanation is that physical stress of the collar in the first period of time after opening of the package (either during application on the cat or later by the cat itself) can cause a high release of propoxur. To some degree this will always occur during application, because collars often are somewhat difficult to tighten, needs to be adjusted in length, the animal does not stand still, etc., but in case of an animal being more sensible than others it could possible be the cause of intoxication.

4 Conclusions

From the obtained results of this investigation it can be concluded that rate of release of propoxur from collars is not constant over the recommended three months period, where the collars are claimed to effectively protect an animal against infestation of fleas. Release of propoxur is highest at the beginning of a period of use and gradually declines over the three months period.

Another important conclusion is that a considerable amount of propoxur can be released when handling a new collar (during application on the animal), because collars release propoxur to the surface if they are bended or twisted.

In relation to what has been the cause of intoxication in cases with cats wearing collars impregnated with propoxur, it is likely that one of these two (or both in combination) characteristics of the collars are involved. A complete explanation of the cause of intoxication involves most likely also biological aspects. These have not been included in the present study, but since cases of intoxication after all are very rare, developing of symptoms of intoxication is probably also related to individual differences in animal sensitivity towards propoxur.

5 References

Miljøstyrelsen (1993): Oversigt over godkendte bekæmpelsesmidler. Miljøstyrelsen. 224 s. - Orientering fra Miljøstyrelsen 1.

Slahck, S.C. (1984): Liquid Chromatographic Determination of Propoxur in Technical and Formulated Products: Collaborative Study. J. Assoc. Off. Anal. Chem. 67(3): 497-498.

Appendix I.

Liquid chromatographic determination of propoxur in a piece of collar

1 Method

1.1 Principle

The method is essentially identical to the official AOAC-CIPAC method described by Slahck (1984). The method includes Soxhlet-extraction of propoxur from a piece of collar followed by reversed phase HPLC with UV detection. Acetonitrile is used as extracting solvent.

1.2 Apparatus

HPLC pump, autoinjector, column thermostat, variable wavelength UV detector and integrator or other data acquisition system.

1.3 Chromatographic conditions

Chromatographic column ODS-Hypersil (Bischoff, Germany), 4.6 mm x 250 mm, 5 µm; column temperature 25 °C; mobile phase acetonitrile/water, 60/40; flow rate 1.5 ml/min; injection volume 20 µl; detection wavelength 280 nm.

1.4 Preparation of standard

Stock solutions of standards (three different concentrations) are prepared by weighing 30, 120 and 300 mg propoxur reference standard (Riedel-de Haën) in 50 volumetric flasks. The standards are dissolved in and diluted to volume with acetonitrile. Calibration standards are prepared by diluting 5.0 ml stock solution to 50 ml with acetonitrile.

1.5 Preparation of sample

A piece of collar (length 2.5 - 3.0 cm, weight *ca.* 1.3 g) is transferred into an extraction thimble (22 x 80 mm) and weighed. 100 ml acetonitrile is poured into the round bottom flask of a Soxhlet extractor and extraction is carried out for 6 h. The extract is concentrated to an oily liquid on rotary evaporator and transferred to 50 ml volumetric flask using 30 - 35 ml acetonitrile and diluted to volume with acetonitrile. 5.0 ml sample solution is diluted to 50 ml with acetonitrile before HPLC analysis.

1.6 Determination

Determination is performed by analysing the sample (duplicate injections) in series with three concentrations of calibration standard; standard injections bracketing the sample. A calibration curve is constructed for each series by plotting peak areas against standard concentrations. Identification is done by comparing retention time of sample and standard (external standard). Typical chromatograms of blind (a piece of collar previously extracted for analysis), standard and sample are shown in figure 1.

2 Validation of method

2.1 Linearity

Linearity of the HPLC analysis has been examined by injecting standards (7 different concentrations between 0.03 mg/ml and 1.2 mg/ml) and plotting peak areas against concentrations. A satisfactory linearity (r = 0.9995) was found. Variations in calibration curve slopes between days/series were below 0.7 %.

2.2 Precision

Precision of the method was calculated as the mean relative standard deviation of the total number of replicate measurements performed during method development and application. Precision was calculated both for the analytical method itself and including the variation resulting from preparation and sampling of pieces of collar. For the analytical method a precision of 0.8 % was found and for the total application a precision of 2.1 % was found.

2.3 Recovery

True recovery experiments were impossible to perform because we had no access to pieces of collar, which had been prepared with a known amount of propoxur. To evaluate recovery by adding a known amount propoxur to the surface of a collar would also be misleading, since propoxur in authentic samples is distributed within the matrix. As a consequence recovery was estimated by comparing contents found in 6 collars from freshly opened packages (same batch) with label claimed contents. By assuming the product complied with the label claim an estimated mean recovery of 100.7 % was found.

2.4 Detection limit

Method detection limit (S/N = 3) was calculated from replicate analyses of blinds (pieces of collar from which propoxur has been previously removed by extraction). A detection limit of 0.23 mg propoxur/piece of collar was found corresponding to a content of *ca.* 0.17 % of initial (label claim) content.

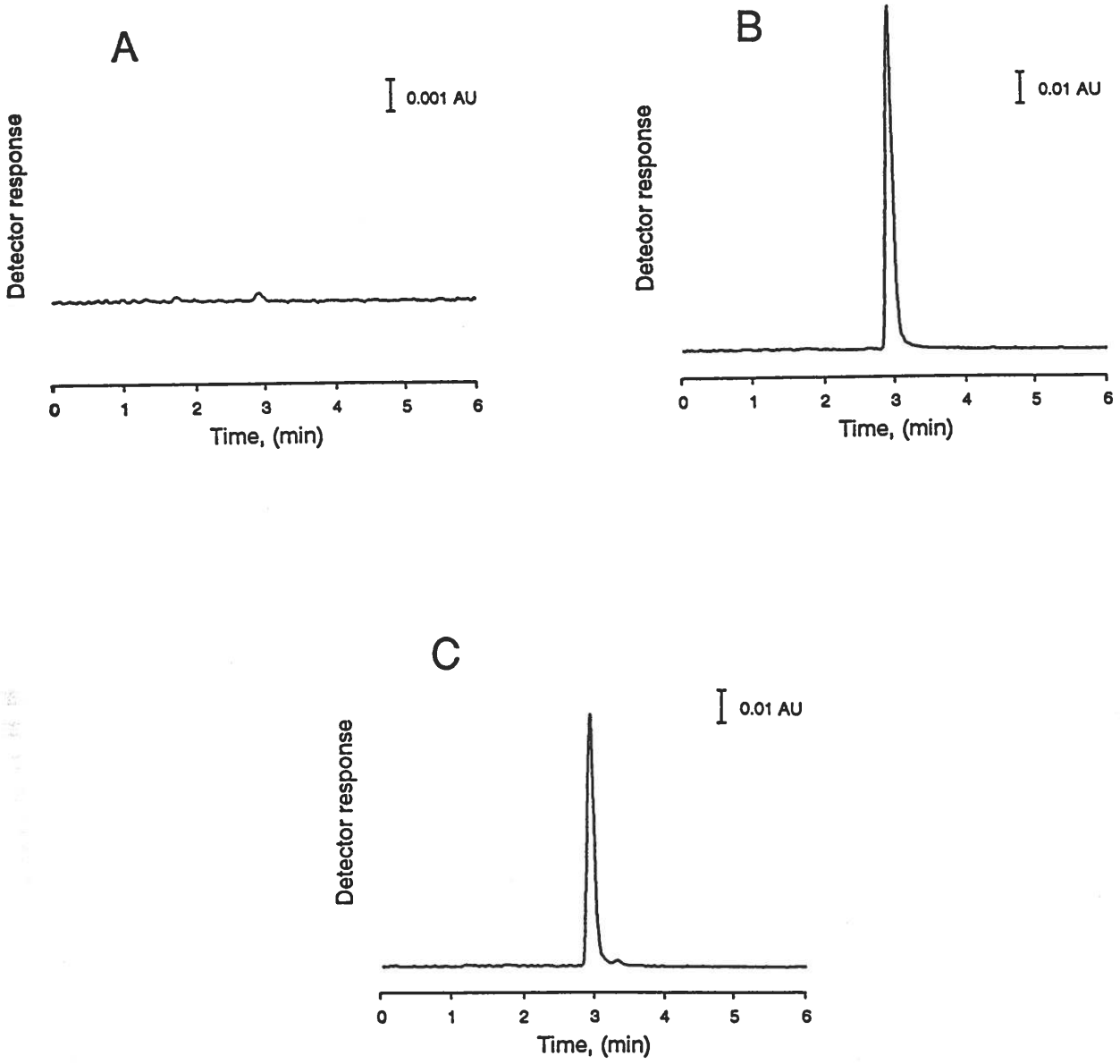

Figure 1. Typical chromatograms from analysis of propoxur in piece of collar. A = blind, B = standard (0.25 mg/ml) and C = sample (propoxur content 7.4 %).

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