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CAUSES AND EFFECTS OF SAMPLE LOSS USING A DRY CAPSULE INJECTION METHOD FOR THE AUTOMATED GAS CHROMATOGRAPHIC ANALYSIS OF PESTICIDE SAMPLES

D. S. LINES, K. R. BRAIN and M. S. F. ROSS

Pharmacognosy Section, Welsh School of Pharmacy, UWIST, Cardiff, Wales (Great Britain)

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SUMMARY

The automatic capsule sampling system is said to have many advantages for accurate gas chromatographic analysis. One is that the solvent may be removed by evaporation before injection, so that no solvent peak is obtained to interfere with the separation. However, working with aldrin and dieldrin, we have shown that up to 50% of the material can be lost from the capsules by air-drying at normal ambient temperature before injection. These losses occurred only with aldrin and it is suggested that they are connected with its relatively high vapour pressure. Significant amounts of material enter the top third of the capsule which is removed during the sealing process. The losses appear to be due to a combination of adsorption to the capsules, creep-up the capsule walls, co-distillation with the solvent, and direct volatilisation. Silylation of the capsules was quite effective in reducing these losses.

INTRODUCTION

Automatic sampling procedures are now in fairly common use for routine gas chromatographic (GC) analysis. As well as allowing unattended continuous operation they have been shown to enhance accuracy[1]. Different methods of autosampling exist but one of the more recent, the Perkin-Elmer AS41, involves the use of small (7 × 3 mm) aluminium capsules. The sample is loaded into the capsule which is sealed using a special apparatus which seals by cold welding and then removes the top third of the original capsule. The capsule is automatically placed in the injection port, a zirconium thorn pierces the capsule, and the contents are vapourised and swept onto the column in a stream of carrier gas. At the end of the analysis the empty capsule is discarded automatically and the next capsule loaded. This system is undeniably more convenient[2] than manual methods and it allows greater scope in the nature of the materials to be analysed as solvent-free samples can be applied by allowing the solvent to evaporate before sealing.

EXPERIMENTAL AND RESULTS

Non-linear responses to aldrin

During an investigation into cyclodiene insecticide metabolism in plants it was necessary to construct wide-range calibration curves for aldrin and dieldrin using a Perkin-Elmer F30 gas chromatograph with AS41 injection system, and dual flame ionisation detection.

As short analysis times were required for the assay of multiple dilute samples it was necessary to remove the solvent (hexane) by drying for 30 min at room temperature. A calibration curve (1–50 μg) of both aldrin and dieldrin was constructed using a fixed sample volume of 10 μl. Dieldrin samples produced the expected straight line relationship between sample size and peak response (height or area). However, dried aldrin samples repeatedly exhibited a non-linear relationship which is typified by Fig. 1. The response was always linear up to at least 5 μg, it flattened off before becoming linear again from 20–25 μg to 30–35 μg, then flattening off to 50 μg. Although the responses were not exactly reproducible from day to day the shape of the curve was essentially constant.
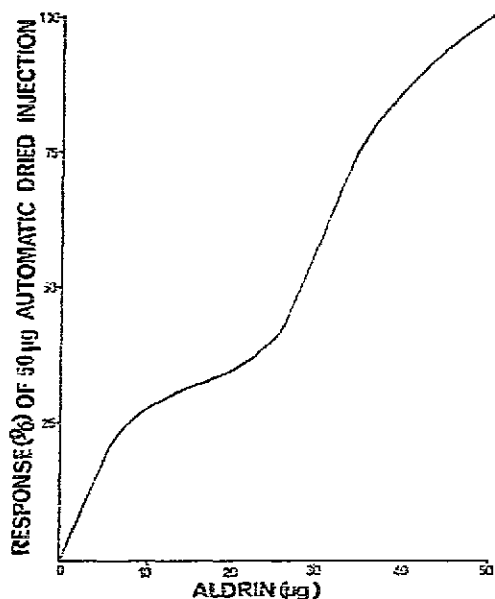

Fig. 1. Typical calibration curve obtained with dried aldrin samples.

Since this peculiar effect had not been reported previously it was investigated further. Initially there seemed to be only two possible causes: (1) an anomalous characteristic in the GC system which was probably enhanced by the autosampling device, or (2) degradation in the capsules or on the column leading to significant losses of material.

Effects of column resilylation and changes in column and running conditions

Initially 6 ft. × 1/8 in. stainless-steel columns of $2\frac{1}{2}$% OV-1 or 3% OV-17 on Chromosorb G AW DMCS (80–100 mesh) were used at 200° and 220°, respectively, with a nitrogen flow-rate of 40 ml/min. These conditions were consistent with maximum resolution. Dieldrin usually had a retention time about twice that of aldrin. All of the metabolic products so far observed run after aldrin.

It was considered worthwhile to re-silylate the columns and to run analyses at higher (250°) and lower (150°) temperatures to encourage or restrict any degradation. Although aldrin is reported[3] to be stable up to 240° it has been suggested[4] that it may decompose under certain conditions. Below 170° (OV-1) and 190° (OV-17) the dieldrin peak tailed badly, whilst above 215° (OV-1) and 240° (OV-17) the retention time of aldrin was too short and separation from dieldrin was insufficient. No alteration in the inflection observed with aldrin was found under any conditions whilst dieldrin retained a straight line relationship. Further tests with other stationary phases in stainless-steel columns gave similar results.

To ensure that the detector was not at fault the column was crossed so that the samples were detected on the second channel but this also had no effect on the response.

Effects of using glass columns and an alternative instrument

Samples were run using manual injection on a Pye 104 gas chromatograph using 5 ft. × 1/4 in. glass columns packed with $2\frac{1}{2}$% OV-1 at 200°. In this case responses to both aldrin and dieldrin were linear. No problems occurred due to solvent peak interference as the columns were of larger bore and separation was enhanced. This suggested that the metal columns in the F30 could be responsible for degradation, although this did still not entirely explain the form of the inflection observed. Alternatively the problem could be associated with the injection system or the presence of solvent.

A series of automatic injections were made on the F30 using a 6 ft. × 1/4 in. glass column of $2\frac{1}{2}$% OV-1 on Chromosorb G AW DMCS (80–100 mesh). These were in the range of 1–50 μg of aldrin and were either made undried (capsule sealed immediately after loading) or dried (solvent allowed to evaporate for 30 min before sealing). The undried aldrin produced a straight line relationship whereas the dried aldrin gave an inflected curve similar to those previously observed. A comparison of the responses of the dried and undried samples showed that even at the higher concentration levels, where the responses to dried samples were almost linear, the responses were significantly lower for the dried samples than those for the undried samples. These losses ranged from almost 0% at 5 μg to over 50% at 50 μg.

Possible methods by which material could be lost

It seemed possible that chemical degradation could occur during drying or after injection and that the presence of solvent inhibited this degradation. On the other hand losses could be due to physical effects associated with drying.

Possible chemical causes. Since no other peaks with longer retention times had been observed any breakdown must have been to smaller molecules, rather than to substituted or polymerised products. The most likely degradation is the retrodiene reaction in which aldrin fragments to norbornadiene and hexachlorocyclopentadiene

(Fig. 2). Dieldrin cannot undergo such a reversal since it would be necessary first to reduce the epoxide ring, a step requiring considerable energy[5]. It would be expected that the retrodiene reaction of aldrin would only occur at high temperature and in an open system[6]. Both of these conditions are met during analysis by the autosampler.
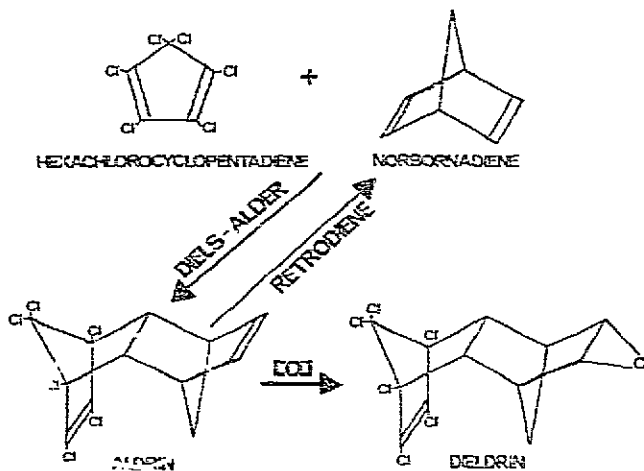

Fig. 2. Synthesis and possible degradation of aldrin and dieldrin.

To determine whether low-boiling degradation products were present after drying the aldrin in the capsules, temperature-programmed runs were made from 50 to 230° with both dried and undried samples. Comparison was made with norbornadiene and hexachlorocyclopentadiene standards and it was found that both of these occurred in the undried samples. This is not unexpected as they are present in small quantities as residues of the starting materials from manufacture[7]. However they were absent from the dried samples, presumably due to volatilisation during drying. Further raising the injection temperature did not induce the formation of these compounds. In an attempt to instigate the retrodiene reaction several dried aldrin samples were heated strongly in open and in closed melting-point tubes and then analysed, but there was still no evidence of breakdown. Furthermore programmed runs from 200–300° showed no evidence of degradation products of longer retention time in these samples. Additionally several capsules were filled and dried before sealing. They were then heated to 200° for one hour before injection. Again no degradation was detected. Finally no appreciable differences were noted when other solvents (acetone and absolute ethanol) were used, or when the drying time was varied, provided that all solvent was removed.

Possible physical causes. It was noted that, only with aldrin, and especially with the larger sample sizes (20–50 μg), the peaks were considerably broader after drying. At first it was thought that this might be due to impurities or conversion products co-chromatographing with aldrin. Under certain conditions acute peak tailing was also evident. It seemed possible that this broadness and tailing could be due to a hold-up in the volatilisation of the sample from the capsule wall in the dried

state. This was supported by the fact that if solvent was put back into the capsule after it had been dried the normal peak shape was obtained. However, the peak after re-addition of solvent was much smaller than that of the original dried sample without solvent, and as little as 10 % of the size of the undried sample peaks of the same load. It was possible that this solvent re-addition caused material to move up the capsule and into the area removed during the sealing process, or, possibly, even further and into the atmosphere. These observations led us to consider the physical properties of aldrin and dieldrin, and in particular their relative volatilities.

When aldrin was first introduced concern was expressed with regard to its relatively high volatility as it has a vapour pressure approximately 100 times greater than that of DDT[8]. Ways were sought to reduce volatility and hence render the material more persistent. The epoxide, dieldrin, has a vapour pressure variously estimated as 24–100-fold less than that of aldrin which has a vapour pressure of about 2.3×10^{-5} mmHg at 20°. Work on the rate of loss of aldrin and dieldrin from glass plates (presumed non-absorptive) using ^{36}Cl-labelled compounds[9] indicated that aldrin was about 15 times more volatile than dieldrin, where the crystals were roughly the same size. Where deposit densities of aldrin exceeded 1 μg/cm^2 the rate of loss was about 3 μg/cm^2 per day and was dependent upon both the size and shape of the crystals and the prevailing conditions. Increased temperature produced the greatest change in the rate of loss, whereas changes in relative humidity were least important. It was calculated that 0.2 cm^2 of capsule wall was covered by the 10 μl loadings of aldrin solution, leaving 0.5 cm^2 of wall area above possibly available for interaction.

Hence, in spite of its high vapour pressure, simple volatilisation of aldrin alone was unlikely to be sufficiently great as to explain all the observed effects. It was, however, possible that the high vapour pressure of aldrin allowed it to co-distill with the solvent. To investigate this possibility several 1-mm melting-point tubes were injected with 10 μl of solutions of aldrin or dieldrin containing 5, 20, and 50 μg material and allowed to dry at room temperature. When the aldrin solution dried out a crystalline ring was observed about 5 mm further up the tube than the original meniscus level of the solution. Dieldrin produced crystals at the original liquid level. This theory of co-distillation could explain the observation that more aldrin was lost from the capsules when they were dried out and then solvent re-added before sealing.

Summary of possible causes of aldrin loss

(1) Retention of aldrin by adsorption onto the capsule wall during drying, this material being retained after injection.

(2) Co-distillation with the solvent which could produce either total loss from the capsule or movement of material into the portion of the capsule lost on sealing.

(3) Creep of the solution up the surface of the capsule into the area removed on sealing.

(4) Direct volatilisation (unlikely to be a major cause).

Investigation of relative importance of suggested physical causes of loss

It was decided to investigate the levels of aldrin left in the capsule clippings after sealing and, if possible, to determine methods of reducing these losses. Peak area estimations were used from this stage because of the disparity in peak shapes already commented upon.

Manual (and therefore undried) aldrin injections allowed the production of a total standard calibration curve (Fig. 3). In subsequent experiments the amount of aldrin retained in the clips of sealed samples was determined by transferring the excised clips to fresh capsules. These were sealed, either with or without the addition of hexane as solvent, according to whether the corresponding capsule was injected dried or undried.
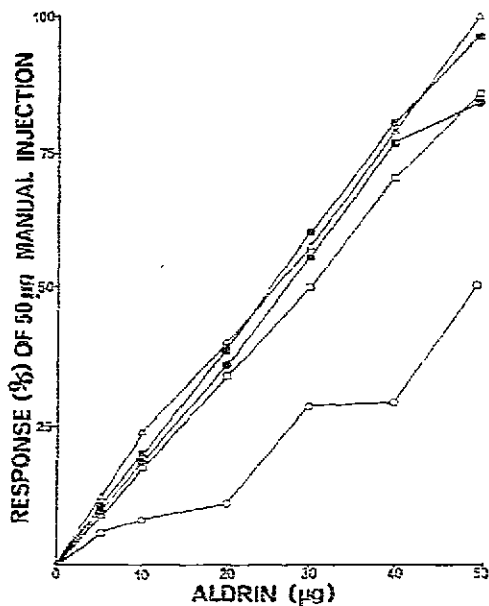

Fig. 3. Comparison of manual, automatic undried and automatic dried injections, which also shows the effect of drying on recovery of aldrin from capsules and clippings. △, Manual injection; ●, automatic injection of undried capsules; ○, automatic injection of dried capsules; ■, sum of automatic injection of undried capsules and of aldrin recovered from clippings; □, sum of automatic injection of dried capsules and of aldrin recovered from clippings.

Two batches of aldrin samples (5–50 μg) were placed in capsules at the same time. They were treated as follows before injection: either (1) sealed undried and the clips placed into fresh capsules with 10 μl solvent, or (2) dried for 30 min at 21° in a draught-free area, sealed, and the clips placed into fresh capsules which were sealed without the addition of solvent.

The relative peak areas of various capsules and clippings are shown in Fig. 3 together with those of the manual undried injections mentioned earlier. A significant amount of material was present in the clippings of the capsules which had been dried, and even when the amounts of aldrin in both the capsule and the clipping had been summated, there was still an appreciable loss unaccounted for. To all intents and purposes the amounts in the capsules and clippings of the undried samples equalled the values produced by manual injection. Henceforth the undried injected totals were taken as 100% and all other data were compared to this. It is worthy of note that some material was evident in the clippings of undried capsules even though sealing had been carried out within 10 sec of placing the solution in the capsule.

Meanwhile further attention was focussed on the unusual peak behaviour of the dried aldrin samples. It was mentioned previously that physical effects were assumed to be responsible. The aluminium capsules have a coating of aluminium oxide on the surface and this was shown by microscopic examination (Fig. 7a) to be quite rough. The capsules were treated as follows during attempts to alter the surface effects which could be associated with the aluminium oxide coat:

(1) Heated to 100° for 1 h to activate absorptive sites on the aluminium oxide;

(2) Treated with 20% HCl for 2 min to dissolve the oxide coat, washed with methanol, and stored in hexane until use to prevent the spontaneous oxidation of the aluminium;

(3) Treated with bis(trimethylsilyl)acetamide for 18 h at 20° and dried at 100° for 1 h to silylate the active centres in the oxide coat and hence reduce adsorption.

With all these capsule treatments the trend was towards a reduction in the peak broadening effect and in tailing. Silylation was most and heating least effective. Only with the silylated capsules there was any indication that less aldrin was lost from the capsule. The reason for the reduced tailing after heating, the converse of what had been anticipated, is unknown. It appears that the surface coating is responsible for the change in peak shape and that adsorption to the walls of the untreated capsules may be one way in which aldrin is lost. When the solvent is replaced in the capsule this adsorption may be reversed, although at the same time the solvent addition may enhance co-distillation losses.

It was found that if the dried untreated capsules were removed from the auto-injector as soon as possible after injection and the residual contents estimated, by macerating batches of ten capsules in 0.5 ml of hexane and re-injecting the solution, as much as 12% of the original load of aldrin could be detected. However if the capsule was left in the injector for a further 7 to 9 min (the normal interval between analyses) then there was no trace of residual aldrin. It is most likely that any aldrin not flushed immediately onto the column would vapourise slowly and be detected only as baseline drift.

Quantitative comparison of aldrin losses from variously treated and untreated capsules

Table I and Figs. 4 and 5 show a composite comparison of the losses of aldrin from untreated and treated capsules under different conditions. Fig. 6 is a schematic diagram indicating the treatment and losses for the 20-μg load. Photographs were taken of the surfaces of capsules to demonstrate the presence of material on the upper wall (Figs. 7a and b). Fig. 6 also gives some data obtained with silylated capsules when the ambient temperature was 4° higher and it can be seen that here the losses were higher. In particular, whereas no unaccounted loss occurred at 21° with silylated capsules, a small unaccounted loss (15%) occurred at 25°. There was also no reversible loss of material at 21° with the silylated capsules, whereas at 25° re-addition of the solvent allowed 6.5% of the material to be recovered. With the untreated capsules, even at 21°, 13.7% was initially lost by drying of which 4.6% was recovered by the re-addition of solvent, leaving 9.1% not accounted for. It must be remembered that these figures for losses are over and above the high amounts left in the clippings.

With the silylated capsules at 25° the amount of material reversibly bound

TABLE I

THE RELATIVE PROPORTIONS OF ALDRIN IN THE CAPSULES AND CLIPPINGS AFTER VARIOUS TREATMENTS COMPARED TO THE SUM OF THE CONTENTS OF THE UNTREATED CAPSULES AND CLIPPINGS INJECTED UNDRIED

GC conditions: column, 6 ft. × 1/4 in. glass, 2½% OV-1 on Chromosorb G AW DMCS (80–100 mesh); nitrogen flow-rate, 70 ml/min; air pressure, 25 p.s.i.; hydrogen pressure, 20 p.s.i.; injector temperature, 200°; column temperature, 200°; detector temperature 250°.

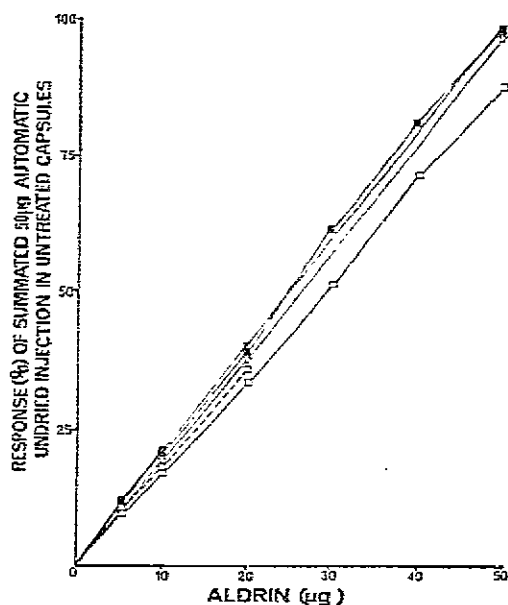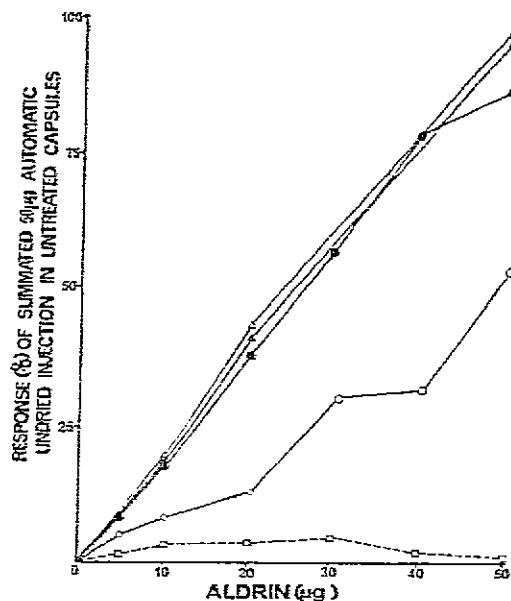| Sample size (µg)	Untreated capsules, undried and injected undried			Untreated capsules, dried and injected dried				Untreated capsules, dried, solvent re-added and injected undried				Silylated capsules, undried and injected undried				Silylated capsules, dried and injected dried			
	In cap (%)	In clip-ping (%)	Total (%)	In cap (%)	In clip-ping (%)	Total (%)	Loss (%)	In cap (%)	In clip-ping (%)	Total (%)	Loss (%)	In cap (%)	In clip-ping (%)	Total (%)	Loss (%)	In cap (%)	In clip-ping (%)	Total (%)	Loss (%)
5	85.0	15.0	100.0	50.0	50.0	100.0	0.0	20.0	70.0	90.0	10.0	—	—	—	—	—	—	—	—
10	90.2	9.8	100.0	38.9	48.6	87.5	12.5	14.6	79.0	93.6	6.4	93.6	6.4	100.0	0.0	97.3	0.0	97.3	3.7
20	92.0	8.0	100.0	27.8	58.5	86.3	13.7	7.7	83.2	90.9	9.1	99.4	0.6	100.0	0.0	100.0	0.0	100.0	0.0
30	93.0	7.0	100.0	48.7	37.3	86.0	14.0	8.2	—	—	—	—	—	—	—	—	—	—	—
40	90.1	9.9	100.0	33.7	49.0	82.7	17.3	1.0	—	—	—	—	—	—	—	—	—	—	—
50	88.6	11.4	100.0	55.6	33.4	89.0	11.0	0.5	—	—	—	96.9	3.1	100.0	0.0	98.0	1.4	99.4	0.6

Fig. 4. Comparison of amounts of aldrin recovered from untreated and silylated capsules. ●—●, Untreated capsules injected undried; ○—○, untreated capsules injected dried; ▲—▲, silylated capsules injected undried; △—△, silylated capsules injected dried; □---□, untreated capsules, dried, solvent re-added and injected undried.

Fig. 5. Comparison of total aldrin recovery from both capsules and clippings of untreated and silylated capsules. ■—■, Sum of untreated caps and clippings injected undried; □—□, sum of untreated caps and clippings injected dried; ▼—▼, sum of silylated caps and clippings injected undried; ▽—▽, sum of silylated caps and clippings injected dried; □---□, sum of untreated capsules and clippings, dried, solvent re-added and injected undried.

stayed more or less constant at about 12–15% throughout the concentration range. These figures are high if it is considered that the silylation process will have removed most, if not all, of the active adsorption sites. The observation that the amount of material which is reversibly bound increases with the load is logical. It was not possible to differentiate between total loss to the atmosphere and irreversible adsorption, but in view of the known effects of silylation on adsorption it is most likely that the material was lost to the atmosphere.

With regard to the capsules at 21° we suggest the following:

(1) With the untreated capsules injected undried the proportion in the clipping was more or less independent of the sample size (10% mean). We assume that this is due to rapid creep of the material up the surface of the capsule wall or by co-distillation with the solvent.

(2) When the untreated capsules are dried a large proportion of the aldrin is found in the clippings (30–60%). In this case there is much more opportunity for creep and co-distillation.

(3) When solvent is placed back into a dried capsule much more aldrin is found in the clippings. As some of the aldrin has presumably been already transferred to the upper part of the capsule it is likely that co-distillation is more important than creep here.
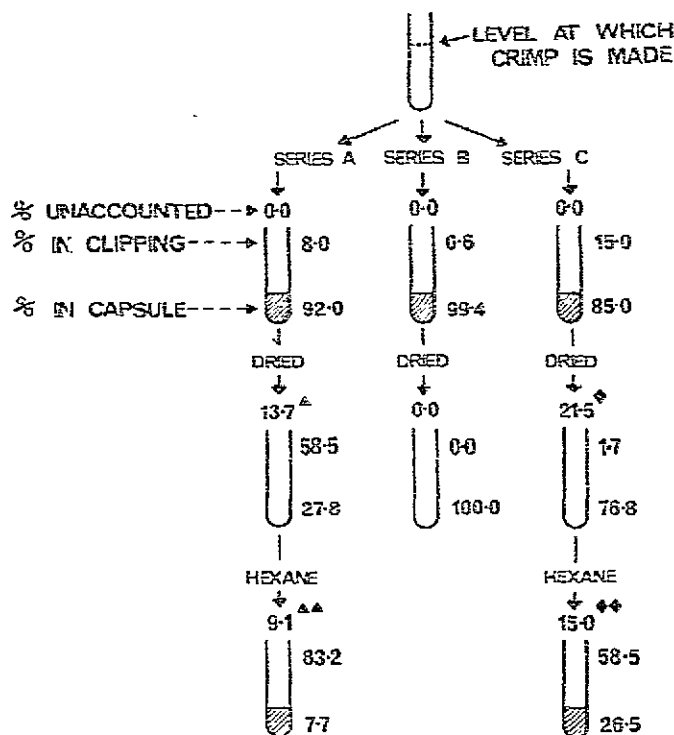
Fig. 6. Schematic of pattern of distribution of aldrin in capsules and clippings and the proportions lost or unaccounted at various stages (all 20 μg injections). Series A, untreated capsules at 21°; B, silylated capsules at 21°; C, silylated capsules at 25°; 13.7▲ − 9.1▲▲ = 4.6% reversibly bound to walls of capsule and clipping, leaving 9.1% unaccounted for. 21.5◆ − 15.0◆ ◆ = 6.5% reversibly bound to walls of capsule and clipping, leaving 9.1% unaccounted for.
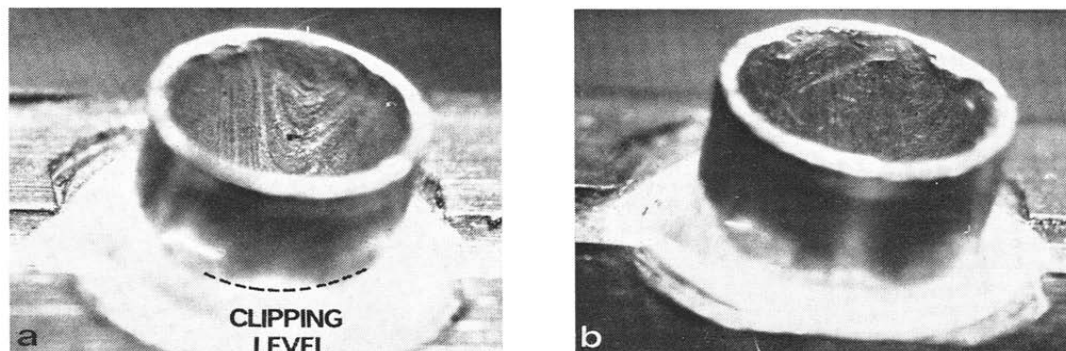

Fig. 7. (a) Untreated capsule, the striations on the capsule wall are clearly visible. (b) untreated capsule, containing 50 μg of aldrin in 10 μl of solvent, allowed to air-dry for 30 min; note the material in the clipping area.

(4) Silylation would be expected to reduce or remove creep effects. Thus aldrin will not only not creep up into the clipping area but will also have a lower surface area for direct volatilisation. However, the results with silylated capsules at 25° suggest that co-distillation may be important at higher ambient temperatures.

(5) The aldrin calibration curve produced for dried silylated capsules was a perfect straight line in contrast to the inflected curve found with the dried untreated capsules. The reasons for this are not clear but it is possible that the losses observed are dependent upon sample size and that at higher levels saturation of the response reducing system occurrs.

(6) Since an examination in less detail with dieldrin did not produce losses it appears that the differences in behaviour are due to the high vapour pressure of aldrin.

CONCLUSIONS

We are able to draw certain conclusions which would seem to limit the applications of the automatic capsule sampling injection system.

(1) It is not possible to inject dried samples of all materials.

(2) It is possible to lose considerable amounts of the sample placed into the capsule, and these losses may occur almost immediately.

(3) A low melting point is not essential for losses to occur as we experienced heavy losses using a material with a melting point of 104°.

(4) With some assays it may be necessary to consider the use of capsules manufactured from other materials (gold capsules are listed but were not available for comparison).

(5) It seems most likely that the observed effect is due to a combination of different processes and that the relative importance of each of these will depend upon the precise conditions.

Finally the authors would like to point out the inadvisability of injecting capsules which contain the clippings of other capsulses as there is a tendency for pieces of aluminium to become lodged in the thorn used to pierce the capsule, with a resultant impairment to carrier-gas flow.

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