TOXLINE/MEDLINE SEARCH - POLYURETHANE

AN-1569

Fd Cosmet. Toxicol. Vol. 6, pp. 329–338. Pergamon Press 1968. Printed in Great Britain

The Migration of Lead from Polymers in the Rat Gastro-intestinal Tract

J. C. GAGE and M. H. LITCHFIELD

Imperial Chemical Industries Limited, Industrial Hygiene Research Laboratories, Alderley Park, Cheshire, England

(*Received 20 October 1967*)

Abstract—The migration of lead from four polymer formulations during their passage through the rat gastro-intestinal tract has been investigated. Diets containing 1% of the formulations have been administered to groups of rats for 4–5 months and measurements have been made of the lead content in blood, bile and urine, and in liver, kidneys and bone. As a reference, similar analyses have been made on groups of rats receiving diets containing 2, 6 or 20 ppm lead as lead nitrate. Lead is poorly absorbed from rat intestine; with the diet containing 20 ppm lead it is not possible to detect a significant increase of lead in blood, urine or liver. A significant rise has been observed in kidney and bone, however, and bile is probably the best index of lead absorption. The greatest migration of lead into the gut from the polymer formulations was observed with PVC containing 2·3% lead sulphate, where the amount was equivalent to about 3 mg lead/g polymer. A more realistic assessment of the ingestion risk is obtained from the amount of lead migrating per unit surface area of polymer under these conditions; with the PVC formulation this was in the region of 25 μg/cm^2. The surface area of this formulation ingested daily would have to exceed 40 cm^2 before the daily addition of lead to the diet would exceed 1 mg, and this area would be much larger in the case of the other polymers. It is concluded that the risk of lead poisoning from ingestion of any of the four polymers is negligible.

65K

Please copy the 1st page

INTRODUCTION

From time to time over a number of years there have been reports of cases of lead poisoning in children, thought to derive from the ingestion of dried flakes of lead-containing paints from treated woodwork (Moncrieff, Koumides, Clayton, Patrick, Renwick & Roberts, 1964). These observations, and the allegation that young children are more sensitive to lead than are adults (Greengard, 1966), have led to the suggestion that a tighter legislative control should be exercised on the lead content of surface coatings and of other materials that may be chewed or sucked by children.

An excessively stringent limit on the lead content of such materials would present technical difficulties, as lead compounds provide the basis of cheap and efficient pigments, stabilizers and polymerization catalysts. It is desirable that before any new legislation is drafted, an attempt should be made to ascertain whether current formulations constitute a real hazard. Four typical polymer formulations containing lead have been investigated (Table 1); polythene containing a lead carbonate pearlescent pigment, polypropylene containing a lead chromate-molybdate pigment, PVC stabilized with tribasic lead sulphate and rigid urethane foam catalysed with an organic lead salt.

As a direct toxicological study of the incidence of lead poisoning in experimental animals dosed with these formulations was considered unlikely to provide useful results, an investigation of the migration of lead from these materials as they pass down the gastro-intestinal

Table 1. *Particle size and lead content of polymer formulati[illegible]corporate[illegible]at diets*

Polymer	Particles (%) retained by mesh 36	52	72	Lead component	Lead content In polymer (%)	In diet (ppm)
Polythene	45	31	12·5	Lead carbonate	0·4	40
PVC				Tribasic lead sulphate	2·3	230
Polypropylene	56	32		Lead chromate-molybdate	0·5	50
Rigid urethane foam				Lead 2-ethylhexoate	0·07	7·5

tract was undertaken. In general, studies on the migration of components from polymers involve the use of simulated solvents under conditions which are likely to accelerate the extraction, but as it was not possible to devise with any confidence extracting solvents which would simulate the conditions existing in the various regions of the gastro-intestinal tract, it was decided to study the migration directly by administering the materials in a finely divided form to the diet of experimental animals, and then investigating the amount of lead liberated during passage along the gut.

Rats were used in this investigation and the polymer samples were finely divided and incorporated in the diet at a concentration of 1%. This was regarded as the highest concentration which would not affect the nutritive value or palatability of the diet. It is known that the bulk of lead in the diet is not absorbed but is excreted in the faeces (Kehoe, 1961a), and as it was not possible to devise an analytical method for faeces capable of differentiating between the lead contained within the polymer and that migrating from it, it was necessary to use an indirect approach to the problem. A proportion of the lead in the diet is absorbed into the circulation; part of this is re-excreted into the gut th[illegible]h the bile [illegible]e is stored in tissues and the remainder is excreted in the urine (Cantarow [illegible]rumper, 19[illegible]). The amount of lead in the urine, while not a direct measure of the amount of lead migrating from the ingested polymer, should enable this to be estimated, if it is compared with the urinary excretion of lead by rats on diets with known amounts of added lead. This approach has been followed in this investigation, but as it is difficult to avoid contamination of urine by lead in the diet or faeces, more reliance has been placed on the analysis of tissues and bile in the comparison of groups of rats receiving the polymers or lead in their diets.

EXPERIMENTAL

Preparation of diets. The four polymers investigated were supplied by Imperial Chemical Industries Ltd., Dyestuffs and Plastics Divisions. They were finely ground and incorporated at a level of 1% into standard rat diet, which, after the addition of 12% malt extract and 2% maize oil, was formed into pellets (Clark, McElligott & Weston Hurst, 1966). The lead contents of the samples and of the diets are shown in Table 1. Three further diets were prepared by incorporating lead nitrate into the standard diet to give lead concentrations of 2, 6 and 20 ppm above the lead content of the control diet, which was found by the analytical procedure described below to have an average value of 1·5 ppm. This method was also used to check the lead content of the diets at intervals throughout the experiment.

Animal experiments. Young male albino rats of the Alderley Park SPF strain, initial body weight 105–120 g, were used in these experiments. The diets containing lead nitrate

and the materials under investigation were fed for 4–5 months to groups of 30 rats, and at the same time a group of control rats was maintained on a normal diet. At the start of the experiment several control rats were killed, and the livers, kidneys, bone (femur) and blood were taken from each animal for analysis. Similar measurements were made on a sample of the test and control animals killed at intervals throughout the duration of the experiment. At the end of the experiment a pooled sample of 24-hr urine was collected from five rats on each diet, and from another pair of rats in each group a 24-hr sample of bile was taken by means of a polythene cannula exteriorized at the back of the neck.

The weight of the rats was measured at weekly intervals throughout the experiment, and the food intake of the groups was also recorded.

The determination of lead. The recommendations of the Analytical Methods Committee of the Society for Analytical Chemistry (1960) were followed for the destruction of tissues; the method of Browett & Moss (1965) was used for the oxidation of urine and bile. Liver, kidney and bone were ashed overnight at 500°C, magnesium nitrate being used as an aid for the destruction of liver and kidney tissue. Blood was oxidized by a mixture of nitric and perchloric acids (3:2, by vol.). Diets other than that containing PVC were ashed by the method used for bone. With the PVC diet this procedure involved large losses of lead, but satisfactory results were obtained by oxidizing with a mixture of sulphuric, nitric and perchloric acids (2:1:1, by vol.).

Lead was determined colorimetrically by the dithizone procedure (method A) described by the Analytical Methods Committee of the Society for Analytical Chemistry (1959). Checks for bismuth were made throughout the study and in no case was any interference detected. The smallest amount of lead which could be determined with reasonable precision was 0·3 μg, corresponding to an optical density of 0·01 unit. The amounts of samples taken for analysis and the limit of detection of lead in each are shown in Table 2.

Table 2. *Sensitivity of analytical method for lead in tissues, body fluids and diets*

Sample	Amount taken for analysis	Lower limit of lead detection (μg/g or μg/ml)
Kidney	2 g	0·15
Liver	10 g	0·03
Bone	1·6 g	0·20
Blood	2 ml	0·15
Bile	20 ml	0·015
Urine	20 ml	0·015
Diet	1 g	0·3

RESULTS

The concentration of lead found in the tissues, urine and bile of the test and control rats throughout the experimental period are shown in Tables 3–8. The values for bone, kidneys and liver (Tables 3, 4 & 5) were subjected to a statistical analysis. The standard deviation of the control values was estimated by an analysis of replicate determinations, and the significance of the differences between test and control measurements was assessed, using a control

sample taken at the same time as the test sample. Significant differences ($P \leq 0{\cdot}05$) are marked in the tables with an asterisk. The significance of the differences between the test values and the overall control mean was also calculated, and values with $P < 0{\cdot}05$ are italicized in the tables. This latter procedure does not take into consideration any systematic fluctuations in the control values, but it overcomes the error involved in having the control mean based on very few animals.

On both the test and control diets the rats remained in good condition and no significant differences were observed between the different groups in respect of growth and food intake.

DISCUSSION

The absorption of lead from diets containing lead nitrate

The figures in Table 6 for the urinary excretion of lead indicate that there is no significant difference between the control group of rats and those receiving the diet containing 20 ppm lead. This suggests that the additional lead in the diet either is not being absorbed, or, if absorbed, is being re-excreted into the gut. Urinary excretion cannot, therefore, be used as an index of lead released into the gut.

The concentration of lead in the blood (Table 7) and liver (Table 5) also appears to be unaffected by the concentration of lead in the diet or the duration of the experiment, although the figures for blood are near the limit of the method and are not very reliable. With bone (Table 3), there is an indication of an increased lead content from the lead-containing diets, though on account of the rather high standard deviation this only becomes definite with the 20 ppm diet. There is no evidence of an accumulation of lead in bone after the first week. Significantly high lead concentrations are also seen in the kidneys from animals on the 20 ppm diet (Table 4), and here there is a tendency for the figures to fall as the experiment proceeds, possibly due to a greater food intake/kg body w t with th nger animals and to the absence of accumulation of lead in the kidneys.

The concentration of lead in bile, although lower than in blood, can be determined with more precision as the available volume is greater (Table 8). The 6 ppm diet produces an evident increase in biliary excretion of lead compared with the control, and the bile provides the most sensitive index of lead absorption.

The analytical results give an indication of the fate of dietary lead in the rat, at an average daily food consumption of 20 g. On the control diet the daily intake of lead was approximately 30 μg, and of this about 2% was found in urine and about 1·6% in bile. As there appears to have been no accumulation in the tissues, the remainder may be presumed to have been excreted in the faeces. With the diet containing 20 ppm added lead, giving a total daily intake of about 430 μg, the figures for urine and bile were about 0·1 and 0·2% respectively. These figures suggest that the absorption of lead from the gut is very limited in the rat, and as it is not proportional to the dietary concentration, absorption may be due to a mechanism other than passive diffusion. The low blood concentration maintained is evidence against a higher absorption masked by re-excretion into the gut by a route other than the bile, and it may be deduced that absorption of lead in the rat is less than in man, where gut absorption is stated to be in the region of 10% (Kehoe, 1961b).

The absorption of lead from polymers

Polythene. There is a significant increase in the lead content of bone towards the end of the experiment, but no indication of any increase in the kidneys. The liver shows an early

Table 3. *Lead content of bone (femur) in rats fed diets containing lead for up to 19 wk*

Diet	Lead content (μg/g wet weight) of femurs at wk												
	0	1	2	3	4	5	6	7	8	9	11	12	16	19
Control	0·98 (8)			0·56 (5)	0·43 (2)		0·44 (3)	0·46 (2)	0·39 (3)			0·69 (3)	0·62 (6)	0·53 (6)
With lead: 2 ppm		*1·81* (2)	0·96 (2)	1·01 (2)		0·34 (2)								0·72 (3)
6 ppm		0·59 (2)	*1·6* (2)	0·32 (2)		0·49 (2)								0·25 (3)
20 ppm		*1·84* (1)	1·02 (2)	*1·87** (2)		2·56 (2)		0·96 (2)						*1·73** (3)
With polythene			0·83 (3)				0·78 (3)		0·62 (3)	0·53 (2)	*1·64* (2)			
With PVC			*1·31* (3)				*1·35** (3)		0·56 (3)			*1·19** (3)	*1·03** (3)	
With polypropylene			0·41 (3)		0·70 (3)				0·76 (3)			*1·52** (3)	*1·83** (3)	
With urethane foam			0·45 (1)		0·22 (3)				0·59 (3)			0·88 (3)	0·49 (3)	
												0·56 (3)	0·43 (3)	

Values listed are means of determinations on no. of animals indicated in parentheses.
Values marked with an asterisk differ significantly ($P < 0{\cdot}05$) from those of control samples taken at the same time. Italicized values differ significantly ($P < 0{\cdot}05$) from the overall control mean (0·63 μg/g (38), SD 0·35 μg/g).

Table 4. *Lead content of kidneys of rats fed diets containing lead for up to 19 wk*

Diet	Lead content (μg/g wet weight) of kidneys at wk													
	0	1	2	3	4	5	6	7	8	9	11	12	16	19
Control	0·43 (8)			0·37 (5)	0·20 (2)		0·14 (1)	0·61 (1)				0·24 (3)	0·21 (6)	0·20 (6)
With lead: 2 ppm		0·33 (2)	*0·49* (2)	0·15 (2)		0·31 (2)								0·28 (3)
6 ppm		0·41 (2)	0·30 (2)	0·29 (2)		0·30 (2)								0·31 (3)
20 ppm		0·36 (2)	*0·70* (2)	0·11 (2)		*0·91* (2)								*0·42** (3)
With polythene			0·46 (3)					0·63 (2)		0·52 (2)	0·44 (2)			
With PVC			*0·56* (3)		0·26 (1)		0·33 (3)		0·21 (2)			0·24 (3)	0·30 (3)	
With polypropylene			0·34 (2)		0·49* (3)		*0·61* (3)		0·30 (3)			0·46* (3)	*0·55** (3)	
With urethane foam			0·15 (1)		0·37 (3)				0·22 (3)			0·20 (3)	0·22 (3)	
					0·25 (2)				0·17 (3)			0·15 (3)	0·15 (3)	

Values listed are means of determinations on no. of animals indicated in parentheses.
Values marked with an asterisk differ significantly ($P < 0{\cdot}05$) from those of control samples taken at the same time. Italicized values differ significantly ($P < 0{\cdot}05$) from the overall control mean (0·30 μg/g (32), SD 0·144 μg/g).

Table 5. *Lead content of livers of rats fed diets containing lead for up to 19 wk*

Diet	Lead content (μg/g wet weight) of livers at wk										
	0	1	2	3	4	5	6	7	8	12	16	19
Control	0·20 (7)	0·14 (7)		0·15 (6)	0·17 (2)		0·05 (3)	0·09 (2)	0·07 (3)	0·16 (3)	0·12 (6)	0·10 (6)
		0·18 (2)	0·12 (2)	0·07 (2)		0·20 (2)						0·10 (3)
With lead: 2 ppm		0·10 (2)	0·22 (2)	0·17 (2)		0·14 (2)						0·11 (3)
6 ppm		0·17 (2)	0·12 (2)	0·19 (2)		0·15 (2)		0·08 (2)				0·09 (3)
20 ppm			*0·37* (2)		0·13 (3)		0·15* (3)		0·10 (3)	0·15 (3)	0·11 (3)	
With polythene			*0·37* (2)		0·15 (3)		0·16* (3)		0·06 (3)	0·15 (3)	0·12 (3)	
With PVC			0·16 (3)		0·15 (3)				0·14 (3)	0·18 (3)	0·11 (3)	
With polypropylene			0·20 (3)		0·14 (3)				0·09 (3)	0·10 (3)	0·10 (3)	
With urethane foam												

Values listed are means of determinations on no. of animals indicat. parentheses.

Values marked with an asterisk differ significantly ($P < 0{\cdot}05$) from those of control samples taken at the same time. Italicized values differ significantly ($P < 0{\cdot}05$) from the overall control mean (0·134 μg/g (45), SD 0·067 μg/g).

Table 6. *Lead content of urine of rats fed diets containing lead for 19 wk*

Diet	Lead levels in terminal urine samples (μg/rat/24 hr)			
Control	0·79 (14)	0·66 (13)	0·61 (15)	0·51 (15)
	0·49 (17)	0·36 (9)	0·55 (18)	
With lead: 2 ppm	0·27 (13)	0·42 (20)		
6 ppm	0·31 (15)	0·31 (14)		
20 ppm	0·73 (20)	0·71 (13)	0·48 (10)	0·23 (6)
With polythene	0·55 (7)	0·26 (5)		
With PVC	0·74 (13)	0·67 (12)		
With polypropylene	0·47 (20)	0·39 (13)		
With urethane foam	0·15 (11)	0·17 (16)	0·47 (19)	0·14 (15)

Each result is based on the pooled 24-hr urine from a group of five rats. Figures in parentheses indicate urine volume (ml/rat/24 hr).

increase in lead content over the controls, and there is an appreciable excretion in bile at the end of the experiment. A comparison of these figures with those obtained from the diets containing lead salts, suggests that the lead released into the gut from the polythene corresponds to a dietary concentration of lead between 6 and 20 ppm.

PVC. There is a marked increase in the lead content of kidneys and bone, which appears at the beginning of the experiment and stays fairly constant. There is a slight initial increase in the content of the liver and an appreciable terminal increase in the biliary excretion. The figures suggest that the migration of lead from the PVC corresponds to rather more than 20 ppm in the diet, perhaps somewhere in the region of 30 ppm.

Polypropylene and urethane. None of the figures in Tables 3–8 gives any indication of an increase in lead content over the controls. This is not surprising with the urethane diet, as if all the lead migrated from the polymer it would barely be possible to detect this by analysis of the organs or excreta.

CONCLUSIONS

These experiments have given some indication of the amount of lead which may be released from the polymers during their passage through the gut in a finely divided form, and these amounts are presented in Table 9 as μg lead/g polymer. It would not, however, be realistic to assume that this amount of lead would leave the polymer whatever the size of the piece ingested. The amount of lead migrating is clearly a function of the surface area of the polymer, and in assessing the possible hazard it is necessary to take this into consideration.

The surface area obtained when 1 g of a solid is subdivided into spheres of diameter r is $3/r\rho$, where ρ is the density. The approximate size analysis of the polymers incorporated in the diets (Table 1) leads to a conservative estimate of 120 cm^2/g for their surface area; the actual area will certainly have been greater than this on account of the irregular shape of the particles. The last column in Table 9 indicates the amount of lead leaving 1 cm^2 surface area of polymer during its passage through the gut. These figures provide a basis on which to assess the hazard arising from the ingestion of these materials, if it is assumed that the conditions obtaining in the human gastro-intestinal tract are approximately the same as those which occur in the rat. It must also be emphasized that the results obtained apply only to the particular lead compounds in the particular polymers used, and cannot necessarily be applied to other formulations. The observations that less lead is released from polypropylene containing 0·5% lead as chromate than is released from the polythene containing 0·4%

Table 7. *Lead content of blood of rats fed diets containing lead for up to 19 wk*

Diet	Lead content (μg/ml) of blood at wk																			
	2		4		5		6		7		8		11		12		16		19	
Control							0·09	0·14			0·05	0·07			0·11	0·12	0·11	0·12		
																	0·18	0·18		
With lead: 2 ppm																			0·23	0·24
6 ppm																			0·14	0·14
20 ppm					0·27	0·57			0·07	0·10			0·16	0·16					0·11	0·16
With polythene	0·17	0·17									0·05	0·07			0·20	0·20	0·12	0·16		
With PVC	0·21	0·21									0·05	0·05			0·13	0·15	0·12	0·16		
With polypropylene			0·15	0·15							0·09	0·09					0·07	0·14		
With urethane foam			0·15	0·18													0·07	0·11		

Values listed are determinations on individual rats.

Table 8. *Lead content of bile of rats fed diets containing lead for 19 wk*

Diet	Lead levels in terminal bile samples (mcg/ml)	
Control	29 (22)	28 (18)
With lead: 6 ppm	49 (13)	43 (14)
20 ppm	47 (22)	59 (15)
With polythene	55 (20)	42 (16)
With PVC	96 (20)	114 (23)
With polypropylene	23 (23)	35 (9)
With urethane foam	31 (16)	20 (23)

Values listed are determinations on individual rats. Figures in parentheses indicate volume of bile collected (ml/rat/24 hr).

Table 9. *Extent of migration of lead from polymers in the gut*

Polymer	Approx. equivalent dietary lead concn (μg/g)	Degree of migration of lead from polymer	
		(mg/g)	(μg/cm²)
Polythene	10	1	8
PVC	30	3	25
Polypropylene	<6	<0·6	<5
Urethane foam	<6	<0·6	<5

lead as carbonate may indicate a difference in the properties of the lead salts or a difference in the polymers.

According to the work of Kehoe (1964), the daily ingestion of 1 mg lead over the basic lead content of the diet for long periods, does not lead to dangerous concentrations of lead in the tissues. With PVC, such a daily lead intake would require the daily ingestion of polymer with at least 40 cm^2 surface area, and with the other polymers a much greater area would be required. If ingestion does not occur every day, then the amount required on any one day would be correspondingly larger. As these polymers are indigestible, and will not disintegrate in the gastro-intestinal tract, it is necessary to decide whether an individual could daily masticate enough material to produce a surface area of this order. It can easily be demonstrated that it is impossible by chewing to reduce these polymers to small pieces, with the possible exception of the urethane foam. This latter has, however, such a low lead content that it does not present a hazard. It must be concluded, therefore, that the risk of lead poisoning from these four formulations is negligible.

Acknowledgements—Technical assistance in this investigation was provided by Mr. T. Green. We acknowledge the assistance of Dr. D. G. Clark for the bile-cannulation preparations and of Mr. C. J. Clark (Pharmaceuticals Division) for the statistical analysis of the results.

REFERENCES

Analytical Methods Committee of the Society for Analytical Chemistry (1959). The determination of lead. *Analyst, Lond.* 84, 127.
Analytical Methods Committee of the Society for Analytical Chemistry (1960). Methods for the destruction of organic matter. *Analyst, Lond.* 85, 643.
Bowell, E. V. & Moss, R. (1965). Manual and semi-automatic methods for the determination of the lead content of urine. *Analyst, Lond.* 90, 715.
Cantarow, A. & Trumper, M. (1944). *Lead Poisoning*. Williams and Wilkens Co., Baltimore.
Clark, D. G., McElligott, T. F. & Weston Hurst, E. (1966). The toxicity of paraquat. *Br. J. ind. Med.* 23, 126.
Greengard, J. (1966). Lead poisoning in childhood. *Clinica pediat.* 5, 269.
Kehoe, R. A. (1961a). The metabolism of lead in man in health and disease. I. The normal metabolism of lead. *J. R. Inst. publ. Hlth Hyg.* 24, 81.
Kehoe, R. A. (1961b). The metabolism of lead in man in health and disease. 2. The metabolism of lead under abnormal conditions. *J. R. Inst. publ. Hlth Hyg*, 24, 101.
Kehoe, R. A. (1964). Metabolism of lead under abnormal conditions. *Archs envir. Hlth* 8, 235.
Moncrieff, A. A., Koumides, O. P., Clayton, B. E., Patrick, A. D., Renwick, A. C. G. & Roberts, G. E. (1964). Lead poisoning in children. *Archs Dis. Childh.* 39, 1.

La migration du plomb provenant des polymères dans le tractus gastro-intestinal du rat

Résumé—On a étudié la migration du plomb provenant de quatre polymères de formules différentes dans le tractus gastro-intestinal du rat. Des aliments contenant 1% des substrats ont été administrés à des groupes de rats de 4 à 5 mois et on a déterminé la teneur en plomb dans le sang, la bile et l'urine, ainsi que dans le foie, les reins et les os. Signalons à titre de référence que des analyses similaires ont été effectuées sur des groupes de rats qui recevaient une nourriture contenant 2, 6 ou 20 ppm de plomb, sous forme de nitrate de plomb. Le plomb n'est que faiblement absorbé par l'intestin du rat. Avec une nourriture contenant 20 ppm de plomb, il n'est pas possible d'observer un accroissement notable de la teneur en plomb du sang, de l'urine, ou du foie. On a toutefois remarqué une augmentation significative dans les reins et les os. Le meilleur témoin de l'absorption du plomb est probablement la bile. La plus forte migration du plomb en direction de l'intestin, à partir de ces polymères, a été observée avec du PVC (chlorure de polyvinyle) contenant 2,3% de suifate de plomb. Dans ce cas, la teneur en plomb est l'équivalent d'environ 3 mg par gramme de polymère. Une évaluation plus réaliste du risque de passage du plomb est déduite, dans ces mêmes conditions, de la quantité de plomb par unité de surface du polymère. Avec la formule du PVC, elle se situe aux environs de 25 μg par cm². Il faudrait que l'ingestion quotidienne de cette formule dépasse la surface equivalente de 40 cm² pour que l'addition de plomb au régime dépasse 1 mg/jour. Cette surface serait beaucoup plus grande dans le cas des autres polymères. On en conclut que le risque d'empoisonnement par le plomb est négligeable en cas d'ingestion de l'un quelconque des quatre polymères.

Der Übertritt von Blei aus Polymeren in den Verdauungskanal der Ratte

Zusammenfassung—Der Übertritt von Blei aus vier Polymerpräparaten während ihres Durchgangs durch den Verdauungskanal der Ratte wurde untersucht. Futter mit 1% der Präparate wurde 4–5 Monate lang an Gruppen von Ratten verabreicht und der Bleigehalt in Blut, Galle und Urin sowie in Leber, Nieren und Knochen bestimmt. Zum Vergleich wurden entsprechende Analysen an Gruppen von Ratten durchgeführt, die 2, 6 order 20 ppm Blei als Bleinitrat im Futter erhielten. Blei wird vom Darm der Ratte schlecht resorbiert; bei einem Futter mit 20 ppm Blei ist es nicht möglich, eine wesentliche Zunahme des Bleigehalts in Blut, Urin und Leber festzustellen. Ein wesentlicher Anstieg wurde jedoch in Nieren und Knochen beobachtet, und die Galle ist wahrscheinlich der beste Index der Bleiresorption. Der grösste Übertritt von Blei in den Darm wurde bei Polymerpräparaten gefunden, die aus PVC mit 2,3% Bleisulfat bestanden, so dass deren Bleigehalt etwa 3 mg Blei/g Polymer entsprach. Eine realistischere Einschätzung der Gefahr der Bleiaufnahme wurde aus der Bleimenge erhalten, die unter diesen Bedingungen je Einheit der Polymeroberfläche übertritt; bei dem PVC-Präparat lag diese bei 25 μg/cm². Die Oberfläche dieses täglich aufgenommenen Präparats müsste 40 cm² übersteigen, ehe die tägliche Abgabe von Blei aus dem Futter 1 mg übersteigen würde, und diese Fläche wäre im Fall der anderen Polymere noch grösser. Es ergibt sich daraus der Schluss, dass das Risiko der Bleivergiftung durch Verabreichung eines jeden der vier Polymere vernachlässigt werden kann.

Fd Cosmet. Toxicol. Vol. 6, pp. 339–340. Pergamon Press 1968. Printed in Great Britain

SHORT PAPER

A Note on the Semi-quantitative Estimation of Aflatoxin M_1 in Liquid Milk by Thin-layer Chromatography

B. A. ROBERTS and RUTH ALLCROFT

Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food, New Haw, Weybridge, Surrey, England

(Received 10 April 1968)

Introduction

It is now well established that cows fed rations containing significant amounts of aflatoxin B_1 excrete in their milk a metabolite, aflatoxin M_1, the toxicity of which is of the same order as that of aflatoxin B_1 for ducklings (Allcroft & Carnaghan, 1963; van der Linde, Freas, de Iongh & Vles, 1964; de Iongh, Vles & van Pelt, 1964; Holzapfel, Steyn & Purchase, 1966; Allcroft & Roberts, 1968). Methods for extracting this toxic metabolite from dried milk have been in use for some time and have recently been compared by Purchase & Steyn (1967). The only recorded method of which we are aware for assaying the aflatoxin content of liquid milk is that described by van der Linde *et al.* (1964). In this method, methanol is used for precipitation of protein and initial extraction of aflatoxin, followed by extraction with chloroform and examination of the combined extracts by thin-layer chromatography. In our hands this procedure has frequently resulted in incomplete precipitation of protein and formation of persistent emulsions, especially when milk samples had been kept in the refrigerator or deep-freeze for several days, or had been reconstituted from dried milk. Moreover, filtration of the methanol-precipitated protein was very slow; the completion of one assay could sometimes take 48 hr.

By using acetone instead of methanol we found that a satisfactory precipitation of protein and extraction of aflatoxin could be obtained. The acetone-precipitated protein allowed rapid filtration, gave little trouble with emulsions and permitted the use of 100 ml samples of liquid milk instead of the 50 ml recommended by van der Linde *et al.* (1964). The use of the larger sample was desirable as we wished to detect the lowest identifiable traces of aflatoxin M excreted in the milk

We have found that the following procedure affords a relatively simple and rapid means of screening samples of liquid milk for the presence of aflatoxin M_1.

Method

Precautions should be taken at all stages to avoid undue exposure to light.

Extraction. Mix 100 ml liquid milk with 200 ml acetone in a 500 ml beaker and bring to boiling point on a water bath. Cool for about 15 min to allow the precipitate to settle, and filter through a 15-cm Whatman no. 41 filter paper. Wash the precipitate twice with 50-ml portions of 70% acetone, combine the filtrates and remove the acetone by evaporation under reduced pressure on a steam bath until only about 100 ml aqueous extract remains. When cold transfer the extract to a 250-ml separating funnel with 50 ml methanol and shake with two successive 100-ml portions of petroleum ether to remove the fat. Extract the aflatoxin