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ORIGINAL ARTICLE

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Extraction of gunshot residues from the larvae of the forensically important blowfly *Calliphora dubia* (Macquart) (Diptera: Calliphoridae)

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Abstract Whole body concentrations of lead (Pb), barium (Ba) and antimony (Sb) were determined in larvae of the blowfly *Calliphora dubia* (Macquart) (Diptera: Calliphoridae) removed from a piece of beef shot and contaminated with gunshot residue and compared with the concentrations detected within larvae feeding on a control piece of beef. Whole larvae were taken into solution and analysed using inductively coupled plasma-mass spectrometry (ICP-MS). Significantly higher concentrations of Pb, Ba and Sb were detected within the larvae feeding on the shot piece of beef compared with larvae that were feeding on the control piece of beef. Initial results indicate that the concentrations of Pb and Sb within the larvae decrease as the duration of feeding increases, whereas Ba concentrations appear to increase, suggesting a bioaccumulation of Ba within the larvae. The second part of this experiment investigated the depuration of Pb, Ba and Sb from the larvae following removal of the gunshot residue source. A significant reduction in Pb, Ba and Sb concentrations within the larvae was observed following the transfer of larvae from the shot piece of beef to the control piece of beef.

Keywords Forensic entomology · Entomotoxicology · Gunshot residue · Inductively coupled plasma-mass spectrometry · *Calliphora dubia*

Introduction

One of the most important considerations for death scene investigators is to establish the cause of death. This may be extremely difficult with severely decomposed corpses and even with only moderately or slightly decomposed corpses. The association of necrophagic insects with decomposing corpses can often provide useful information regarding the likely cause of death. (Goff et al. 1994; Goff 2000; Byrd and Castner 2001). In cases where a decomposed corpse is found, the traditional methods of ascertaining cause of death by chemical analysis can be complicated if solid tissues are unavailable or not suitable for analysis (Beyer et al. 1980; Goff et al. 1997; Goff 2000; Bourel et al. 2001; Byrd and Castner 2001; Gagliano-Candela and Aventaggiato 2001; Introna et al. 2001). As a consequence, there has been increased interest in the use of alternative sources or specimens for chemical analysis. During the past decade some of these alternate sources have involved the application of toxicological analysis to carrion feeding insects in order to identify toxins, drugs or chemicals present on or within the carrion (Introna et al. 2001).

The accurate identification of wounds on decomposed bodies is a very difficult task and analysis by visual means alone is fraught with difficulties that can lead to misinterpretation, especially given that larval activity within a wound generally leads to the destruction of diagnostic features. Larval activity on a corpse may also produce postmortem artifacts that may simulate wounds (Pollak and Reiter 1988), further complicating interpretation. Typically, x-rays of discovered remains are taken and this may lead to the detection of projectiles (bullets) or fragmented projectile pieces, which may be used as evidence for the presence of gunshot injuries (Rainio et al. 2001). The analysis of skeletal remains can often reveal evidence of gunshot injuries if the bullet has left markings on the bones as it travels through the body. These markings are not only useful in identifying gunshot injuries, but also in determining the direction of the bullet passage through the body (Druid 1997).

However, with decomposing corpses and in the absence of these identifying marks within the corpse, other techniques need to be utilised in order to establish whether a firearm was involved in the death.

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The formation and chemical composition of gunshot residues (GSR) and their importance in forensic investigation is covered extensively by Meng and Caddy (1997) and Romolo and Margot (2001).

In this study an investigation has been undertaken into the detection and retention of gunshot residues in the blowfly larvae of *Calliphora dubia* (M) (Diptera: Calliphoridae), a forensically important blowfly species in south-western Australia (Dadour et al. 2001).

Materials and methods

Shooting procedure

Three 1 kg pieces of beef (all from the same animal) were placed on Styrofoam trays on the ground, approximately 50 cm from the end of the firearm muzzle, and each was shot twice. Following shooting, powder tattooing was clearly evident on the surface of the beef around the entrance hole, indicating that unburned powders were exiting the muzzle of the gun and impacting on the surface of the pieces of beef. After shooting, the pieces of beef were placed inside plastic containers, sealed and labeled. At the time of shooting, the wind speed was less than 5 km per hour.

All ammunition used to shoot the pieces of beef were from the same box and fired from the same firearm, a .38 caliber Smith and Wesson six shot revolver. The ammunition used was .38 calibre centrefire ammunition that had been reloaded by the Western Australian Police Service.

Blowfly colonies

The blowfly species used in this experiment was *Calliphora dubia* (Macquart), which is a primary strike species in the south west of Western Australia (Dadour et al. 2001). A laboratory colony of adult *C. dubia* is maintained at the University of Western Australia in a controlled temperature environment at 24°C±1°C with a photo-period of 12 h light and 12 h dark (12L:12D). This blowfly species is viviparous and it is from this colony that adult flies were allowed to larviposit onto the pieces of beef.

Substrate preparation

In experiment 1, two pieces of beef both shot by the revolver and one control piece of beef (not shot) were used. Larvae were placed on the shot and control pieces of beef, and according to the sampling protocol outlined, were removed and analysed for the presence and concentration of Pb, Ba and Sb.

In experiment 2, a second control piece of beef (not shot) was divided up into smaller pieces and used to facilitate the transfer of larvae from one of the shot pieces of beef. For future reference, this control piece of beef used for the transfer of larvae will be referred to as the "transfer beef". This experiment was designed to test for the retention of GSR's over time. Larvae were removed from the transfer beef, according to the sampling protocol below, and the concentration of Pb, Ba and Sb compared with those detected within larvae removed from the shot and control pieces of beef.

Larvae were obtained by placing a few small control pieces of beef into cages of adult *C. dubia* for several hours to allow them to larviposit. Approximately 200 larvae were placed on the first piece of beef containing gunshot residues (GSR#1) and the control piece of beef, whereas approximately 300 larvae were placed on the second piece of beef containing gunshot residues (GSR#2) in order to facilitate the transfer of larvae to the transfer beef. The pieces of beef (on styrofoam trays) were then placed on a 3 cm layer of dry sand inside a square plastic container, covered with mesh and sealed. The larvae were allowed to feed in a controlled temperature room at a temperature of approximately 24°C±1°C, with a photoperiod of 12 h light and 12 h dark (12L:12D).

Sampling protocol

Experiment 1

Larvae were allowed to develop on the respective pieces of beef for 48 h prior to sampling in order for them to reach 2nd instar. Following 48 h of feeding, 6 larvae were removed from each of the 3 pieces of beef (GSR#1, GSR#2 and control). This procedure was repeated at 12 -h intervals until all larvae had left the pieces of beef to pupate. Larvae removed from the shot pieces of beef were sampled from as close as possible to the bullet entrance site, six post-feeding larvae (no longer feeding on the substrate), pupae, empty puparia and emerged adults were also recovered for analysis. Larvae samples were killed in boiling water, strained and preserved in 70% alcohol. Pupal samples were preserved by piercing the anterior end with a fine pin and placed directly in 70% alcohol. The empty puparia and adults were stored in separate ventilated jars.

Experiment 2

Larval samples were transferred from a shot piece of beef (GSR#2) to a control piece of beef in order to determine the depuration rate of Pb, Ba and Sb from the larvae. Following 12 h of feeding on the GSR#2 substrate 60 larvae were transferred to a small piece of transfer beef. This transfer of larvae from the GSR#2 substrate to a new piece of the transfer beef continued every 12 h until all larvae had left GSR#2 to pupate. The number of larvae transferred decreased by 5 every 12 h (ie 55 were transferred after 24 h on GSR#2, 50 after 36 h etc). Larvae that were transferred from GSR#2 were removed from as close as possible to the bullet entrance site.

Larvae were allowed to feed on the respective transfer beef for 12 h before specimens were removed for analysis, six larvae were then removed every 12 h until all larvae had left the transfer beef to pupate. Six post-feeding larvae, pupae, puparia and adults from each piece of transfer beef were recovered for analysis. All samples were preserved by the aforementioned methods.

Instrumentation and operating parameters

A Fisons/VG Plasma Quad 3 Turbo Plus ICP-MS unit, incorporating micro-skimmers was used for the measurement of Pb, Ba and Sb concentrations in larval samples. The analytical protocol for each sample involved a 60 s uptake period, then a 60 s data acquisition period followed by a 90 s washout phase.

The operating conditions for the ICP-MS vary on a daily basis. However the general operating conditions for the ICP-MS during the period of experimentation are outlined in Table 1.

Digestion procedure

Prior to weighing the larvae, each specimen was thoroughly rinsed with ultra pure MilliQ (18MegΩ) water, in order to remove any

Table 1 ICP-MS operating conditions

Condition	Value
RF power	1550 Watts
Gas flow rates	Cool gas 13.5 l min^{-1}
	Auxiliary gas 0.90 l min^{-1}
	Nebuliser gas 0.80 l min^{-1}
Sample uptake rate	0.8–0.9 mL min^{-1}
Nebuliser	Meinhard nebuliser (axial)
Sampling cone	Nickel, 1.0 mm orifice
Skimmer cone	Nickel, 0.7 mm orifice
Lens voltages	Optimised daily
Monitored ions	m/z: ^{103}Rh, ^{121}Sb, ^{123}Sb, ^{137}Ba, ^{138}Ba, ^{191}Ir, ^{193}Ir, ^{206}Pb, ^{207}Pb, ^{208}Pb

excess GSR or debris clinging to the cuticle of the larvae, then dried on absorbent paper towel. Larvae were placed into separate, cleaned Teflon beakers (one larva per beaker) which had been accurately weighed to four decimal places. The increase in mass, due to the larvae, was then calculated by the same process. Five replicates out of the six larvae collected were analysed for each sample period.

An aliquot of 5 ml of re-distilled nitric acid (HNO_3) was added to each Teflon beaker and a Teflon watch glass placed over each beaker. The beakers were placed on a hotplate at 150°C and allowed to reflux for 2–3 h to completely digest the larvae. After this time, the watch glasses were removed from the beakers and the contents reduced to dryness (~3–4 h). The resulting residue was resuspended by the addition of 2 ml of a 4:1 mixture of nitric and perchloric acids (4:1 $HNO_3/HClO_4$) to each beaker and replaced on the hotplate at 150°C for 3 h, or until samples had reached dryness. Once the samples had reached dryness, 50 µl of re-distilled HNO_3 and a few drops of MilliQ water were added to each beaker, using a micropipette, and the residue solubilised. The solution from each Teflon beaker was transferred to a separate acid-washed 15 ml centrifuge vial and the solution made up to 1 ml with MilliQ water, 4 ml of a rhodium (^{103}Rh) and iridium (^{191}Ir, ^{193}Ir) internal standard was added to each vial to facilitate instrumental drift correction. Calibration of the ICP-MS was performed using 1, 2, 5, 10 and 20 ng ml^{-1} multi-element standards, prepared in a 2% HNO_3 solution. Reagent blanks were also prepared by performing the same digestion procedure without the addition of larvae samples.

Statistical analysis

The concentrations of Pb, Ba and Sb detected within the blowfly larvae removed from GSR #1 and GSR #2 were compared with the concentrations within the larvae removed from the control piece of beef. The data were analysed according to Zar (1984) using a one-way analysis of variance test with a 95% confidence interval and significance determined at $p<0.05$.

Results

Experiment 1

Controls v GSR larvae

The larvae that had been feeding on GSR#1 and GSR#2 for 48, 60, 72, 84 and 96 h all contained significantly higher ($p<0.05$) concentrations of Pb, Ba and Sb than those observed within larvae feeding on the control piece of beef (Tables 2 and 3, Figs. 1, 2 and 3). The only exception was for Sb concentrations within larvae that had been feeding on GSR#1 for 72 h (Table 2, Fig. 2).

Although the larvae that had been feeding on GSR#2 showed slightly higher concentrations of all three elements for each time period than larvae feeding on GSR#1 (Figs. 1, 2 and 3), only Ba concentrations were significantly higher. The variation that was observed within the five replicate larvae, sampled at each time period from the control piece of beef, was negligible and consistently low concentrations of each element were detected. The amount of variation within the five larvae sampled from GSR#1 and GSR#2 was greater than the variation within the larvae removed from the control piece of beef (Figs 1, 2 and 3).

The concentration of Sb detected within larvae that had been feeding on GSR#1 and GSR#2 appeared to decrease as the duration of feeding on these pieces of beef increased (Fig. 2). Similarly, the concentration of Pb showed a slight decrease from larvae that had been feeding for 48 h compared to larvae that had been feeding for 72 h. However, following 72 h of feeding the concentration of Pb within the larvae gradually increased during the feeding experiment (Fig. 1). The concentration of Ba within larvae feeding on GSR#1 and GSR#2 remained virtually constant throughout the duration of the feeding experiment (Fig. 3).

Table 2 Analysis of the concentration of Pb, Ba and Sb detected within the *C. dubia* larvae removed from the GSR#1 piece of beef versus larvae removed from the control beef. Five replicate larvae each were analysed from each time period from GSR#1 and from control

Controls v. GSR#1 Sample type/ time feeding	Lead (Pb)	Barium (Ba)	Antimony (Sb)
Larvae – 48 h	F=26.89*	F=87.66*	F=47.92*
Larvae – 60 h	F=27.29*	F=50.27*	F=53.39*
Larvae – 72 h	F=14.33**	F=60.17*	F=2.57, NS
Larvae – 84 h	F=26.45*	F=92.25*	F=9.60**
Larvae – 96 h	F=13.71**	F=34.25*	F=11.14**
Larvae – post-feeding	F=29.39*	F=46.75*	F=0.08, NS
Pupae	F=5.61**	F=15.69**	F=2.14, NS
Puparia	F=1.11, NS	F=2.52, NS	F=4.21, NS
Adults	F=20.83*	F=1.89, NS	F=0.69, NS

Degrees of freedom 9 for all.
*$p<0.003$.
**$p<0.05$.
NS: Not Significant.
F F-statistic value.

Table 3 Analysis of the concentration of Pb, Ba and Sb detected within the *C. dubia* larvae removed from the GSR#2 beef versus larvae removed from the control beef. Five replicate larvae each were analysed from each time period from GSR#2 and control

Controls v. GSR#2 Sample type/ time feeding	Lead (Pb)	Barium (Ba)	Antimony (Sb)
Larvae – 48 h	F=64.37*	F=743.86*	F=120.13*
Larvae – 60 h	F=33.03*	F=162.13*	F=341.27*
Larvae – 72 h	F=31.56*	F=154.82*	F=15.85**
Larvae – 84 h	F=26.77*	F=43.22*	F=7.10**
Larvae – 96 h	F=25.08*	F=43.01*	F=2.66, NS
Larvae – post-feeding	F=9.03**	F=19.87*	F=1.13, NS
Pupae	F=0.76, NS	F=8.67**	F=0.67, NS
Puparia	F=10.91**	F=6.33**	F=0.42, NS

Degrees of freedom 9 for all.
*$p<0.003$.
**$p<0.05$.
NS Not significant.
F F-statistic value.

Experiment 2

Transfer larvae

Following transfer of the larvae from GSR #2 to the transfer beef the concentrations of Pb, Ba and Sb detected within the larvae decreased considerably and returned to concen-

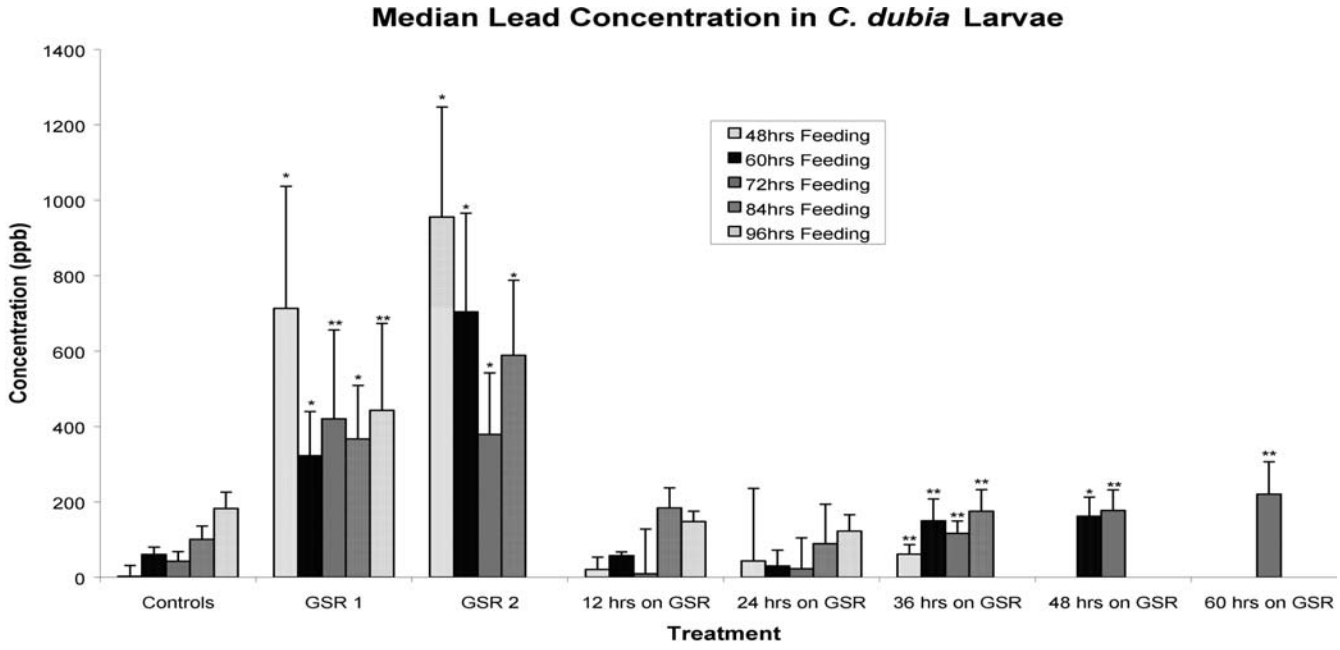

Fig. 1 Median lead concentrations within *C. dubia* larvae, showing the variation in concentrations between larvae removed from the control, GSR and transfer beef. Concentrations are the median concentrations detected within five replicate larvae samples. (*p<0.003, **p<0.05). The columns represent the total time feeding (i.e. the time on the GSR beef, plus the time on the transfer beef)

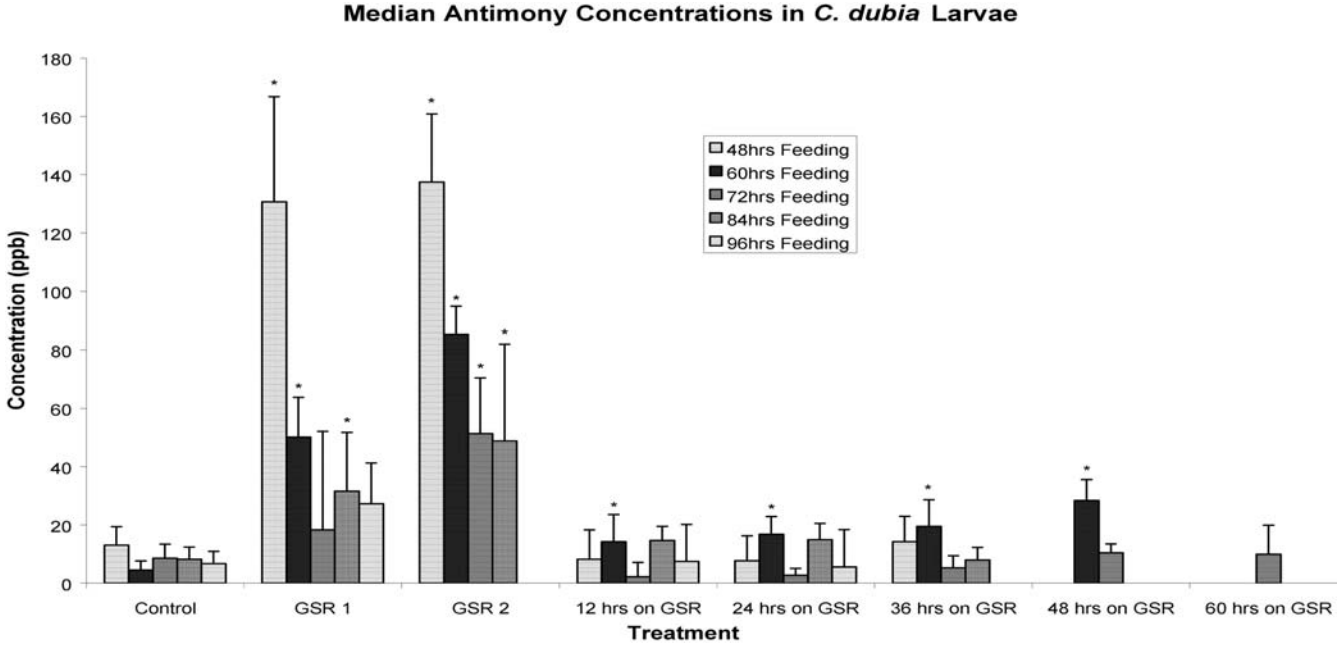

Fig. 2 Median antimony concentrations within *C. dubia* larvae, showing the variation in concentrations between larvae removed from the control, GSR and transfer beef. Concentrations are the median concentrations detected within five replicate samples. (*p<0.003, **p<0.05). The columns represent the total time feeding (i.e. the time on the GSR beef, plus the time on the transfer beef)

trations that were similar to those detected within the larvae removed from the control pieces of beef (Figs. 1, 2 and 3).

Generally, the concentrations of Pb, Ba and Sb detected within the larvae following transfer from the shot piece of beef to the transfer beef were not significantly different to the concentrations detected within the larvae from the control piece of beef (Table 4). There were a few exceptions to this, particularly Pb and Ba concentrations within larvae that had been feeding on the shot piece of beef for 36 h or more (Table 4; Figs. 1, 2 and 3). The concentra-

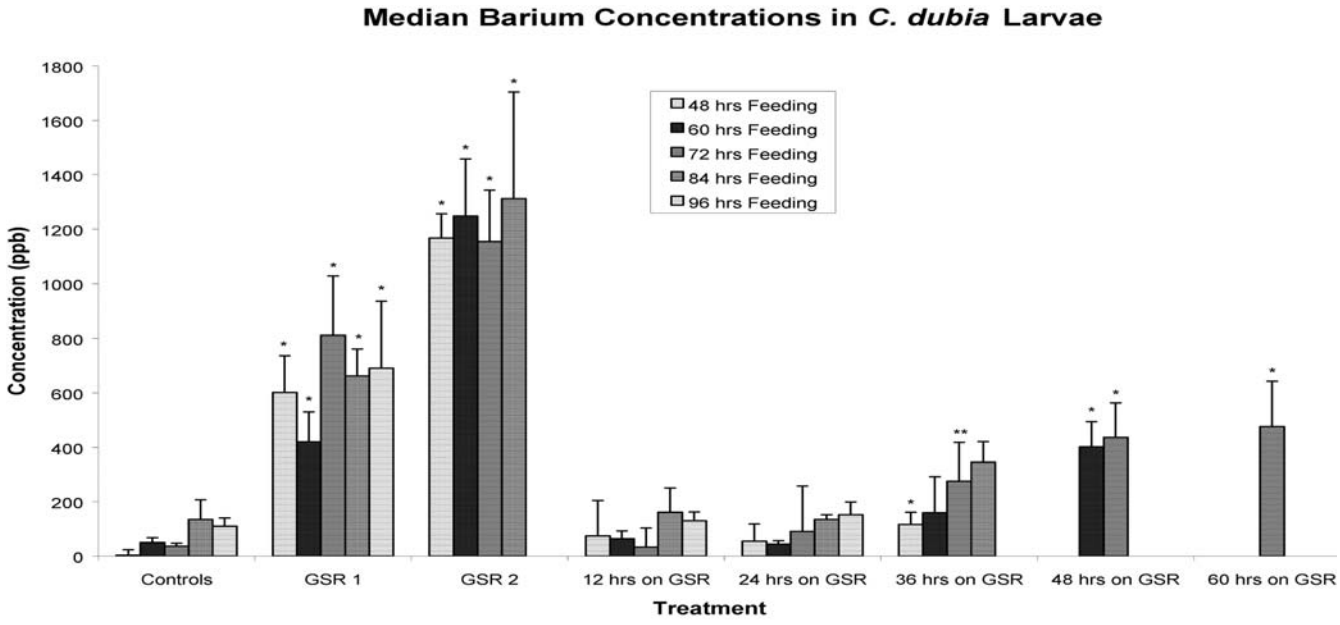

Fig. 3 Median barium concentrations within *C. dubia* larvae, showing the variation in concentrations between larvae removed from the control, GSR and transfer beef. Concentrations are the median concentrations detected within five replicate samples. (*p<0.003, **p<0.05). The columns represent the total time feeding (i.e. – the time on the GSR beef, plus the time on the transfer beef)

tions detected within these larvae were significantly lower (p<0.05) than those detected within the larvae removed from GSR#2.

Post-feeding larvae, pupae, puparia and adults

The post-feeding larvae and pupae removed from both GSR#1 and GSR#2 contained significantly higher concentrations (p<0.05) of Pb and Ba compared with post-feeding larvae and pupae removed from the control piece of beef (Tables 2 and 3, Figs. 4 and 6). However, Sb concentrations in post-feeding larvae and pupae removed from the shot pieces of beef were not significantly different to the Sb concentrations within post-feeding larvae and pupae removed from the control piece of beef (Tables 2 and 3, Fig. 5). The concentrations of Pb and Ba within the post-feeding larvae and pupae removed from the transfer beef quickly decreased to concentrations closely resembling those present in larvae removed from the control piece of beef (Figs 4 and 5). However, significantly higher concentrations of Pb and Ba (p<0.05) were detected within post-feeding larvae and pupae specimens that had been feeding for 48 h or more on the shot piece of beef

Table 4 Analysis of the concentration of Pb, Ba and Sb detected within the *C. dubia* larvae removed from the transfer beef versus larvae removed from the control beef. Five replicate larvae were analysed from each time period

Control larvae v. transfer larvae Time feeding (h)		Lead (Pb)	Barium (Ba)	Antimony (Sb)
12 h on GSR#2	36 h off	F=0.24, NS	F=3.68, NS	F=0.19, NS
	48 h off	F=0.04, NS	F=0.30, NS	F=6.66**
	60 h off	F=0.53, NS	F=1.15, NS	F=2.05, NS
	72 h off	F=4.94, NS	F=0.79, NS	F=0.95, NS
	84 h off	F=0.32, NS	F=1.89, NS	F=1.72, NS
24 h on GSR#2	24 h off	F=2.43, NS	F=4.28, NS	F=0.30, NS
	36 h off	F=0.19, NS	F=1.63, NS	F=23.64*
	48 h off	F=0.13, NS	F=2.37, NS	F=4.20, NS
	60 h off	F=0.50, NS	F=0.23, NS	F=4.06, NS
	72 h off	F=0.73, NS	F=2.61, NS	F=0.48, NS
36 h on GSR#2	12 h off	F=6.35**	F=21.26*	F=0.11, NS
	24 h off	F=15.65**	F=4.62, NS	F=15.95**
	36 h off	F=15.11**	F=15.67**	F=0.33, NS
	48 h off	F=9.27**	F=9.38**	F=0.36, NS
48 h on GRS#2	12 h off	F=24.96*	F=65.07*	F=45.27*
	24 h off	F=17.22**	F=41.20*	F=1.04, NS
60 h on GSR#2	12 h off	F=16.83**	F=23.81*	F=1.76, NS

Degrees of freedom 9 for all.
*p<0.003.
**p<0.05.
NS Not significant.
F F-statistic value.

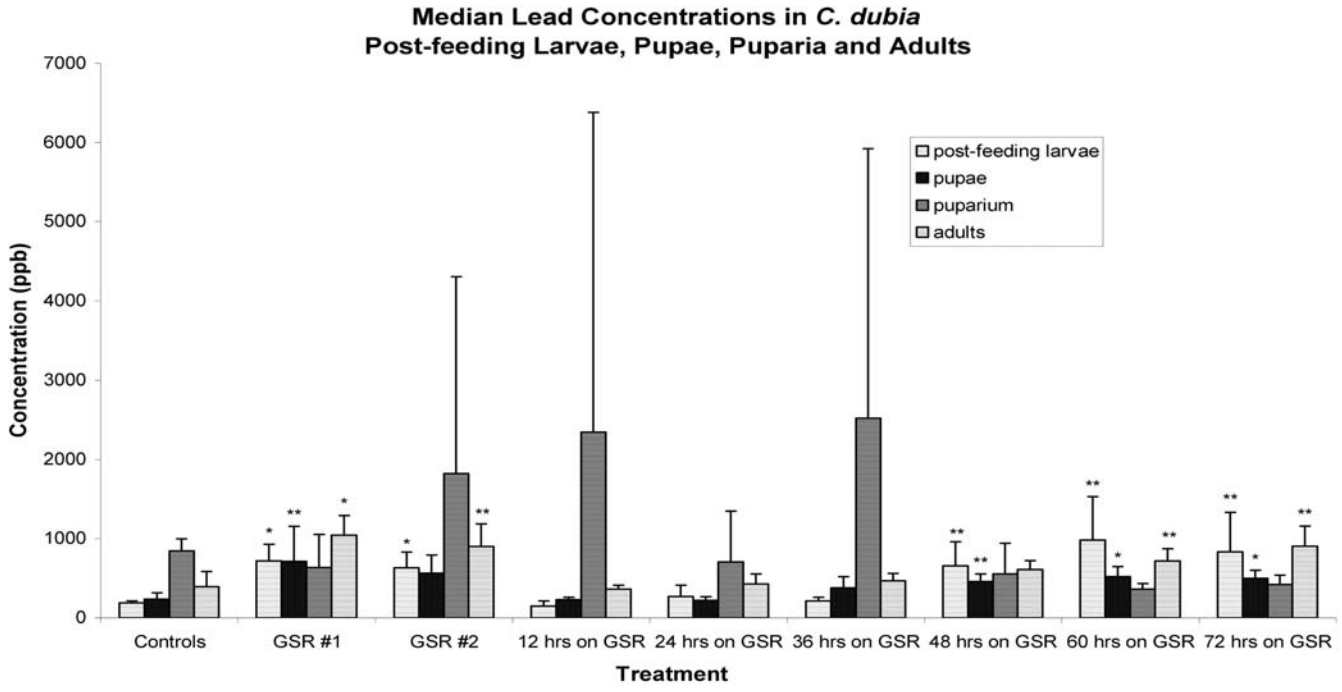

Fig. 4 Median lead concentrations within *C. dubia* post-feeding larvae, pupae, puparia and adults. Concentrations are the median concentrations detected within five replicate samples (*p<0.003, **p<0.05)

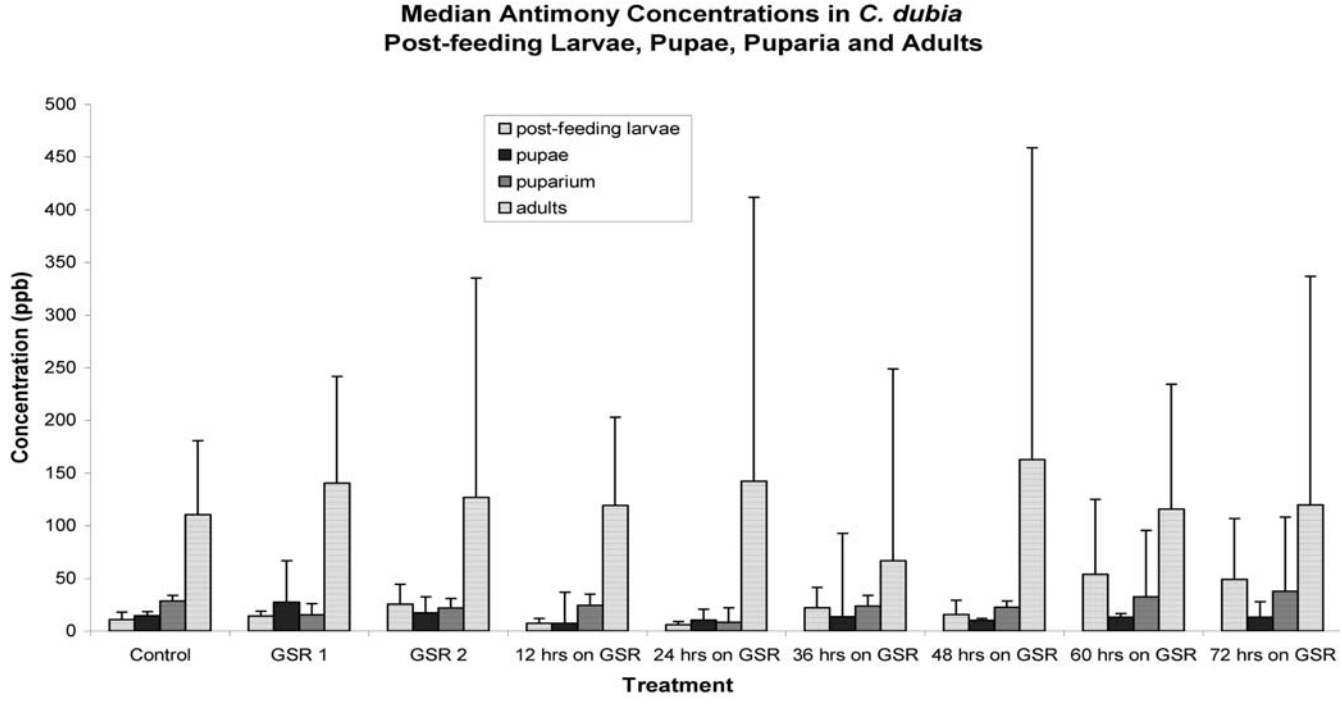

Fig. 5 Median antimony concentrations within *C. dubia* post-feeding larvae, pupae, puparia and adults. Concentrations are the median concentrations detected within five replicate samples (*p<0.003, **p<0.05)

prior to transfer. Unlike the actively feeding larvae, the post-feeding larvae and pupae displayed a high degree of variation in elemental concentration, for all three analytes, within the five replicate specimens (Figs 4, 5 and 6).

Overall, the concentrations of Pb, Ba and Sb detected within the empty puparia of the larvae feeding on GSR#1 and GSR#2 were not significantly different from the concentrations detected within the puparia of the larvae reared on the control piece of beef (Tables 2 and 3, Figs. 4, 5 and 6). Only puparia removed from GSR#2 showed significantly higher concentrations (p<0.05) of Ba (Table 3, Fig. 6). The concentrations of Pb and Ba were relatively

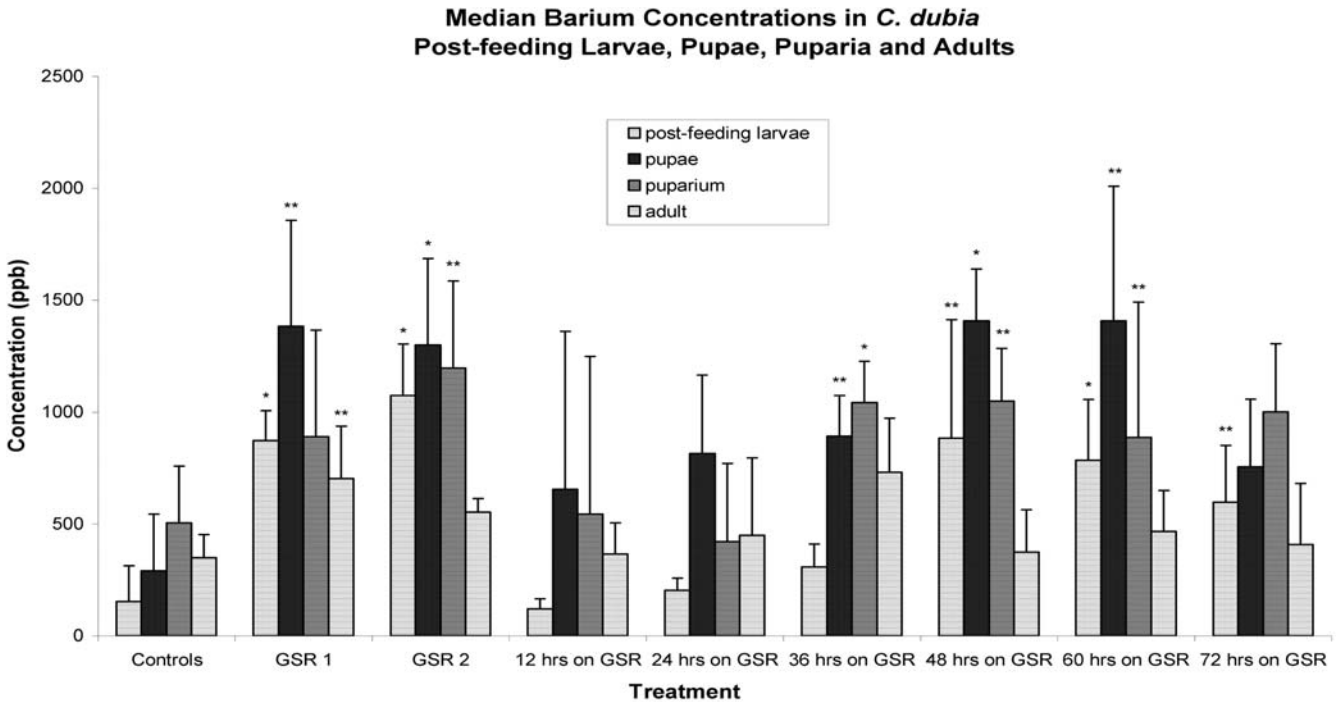

Fig. 6 Median barium concentrations within *C. dubia* post-feeding larvae, pupae, puparia and adults. Concentrations are the median concentrations detected within five replicate samples (*p<0.003, **p<0.05)

high in the puparia from the control piece of beef and a large degree of variation was observed in the concentrations detected within replicate specimens from both the control and shot pieces of beef (Figs 4, 5 and 6).

Discussion

Lead, barium and antimony concentrations were significantly higher in larvae removed from both of the shot pieces of beef when compared with larvae removed from the control piece of beef. Due to the fact that the analysis protocol involved total digestion of the sample to establish whole body concentrations of Pb, Ba and Sb within the larvae, the specific regions where these elements or GSR particles are held and whether they are being incorporated within larval tissues was not determined.

The larvae feeding on the shot pieces of beef showed a gradual decrease in whole body Pb and Sb concentrations as the duration of feeding increased. This may in part be due to an increase in metabolic rate with increasing larval age (Hopkin 1989; Dallinger 1993). Alternatively, this may be an indication of the non-uniform distribution of GSR particles throughout the shot piece of beef. That is, as the period of grazing on the areas containing GSR particles increases, the number of particles available for consumption would ultimately decrease. This non-uniform distribution of GSR particles is also the likely cause of the variation in Pb, Ba and Sb concentrations within the replicate larvae samples.

Unlike the decrease in Pb and Sb concentrations within the larvae with increased feeding time, the concentration of Ba within the larvae removed from the shot pieces of beef, remained relatively constant over the duration of feeding. This suggests that the elimination of Ba from within the larvae is not as efficient as the elimination of Pb and Sb. This may be due to the fact that Ba has similar chemical and physical properties as that of calcium (Ca) which is an essential element required for larval development (Hopkin 1989).

Following transfer of the larvae from the shot piece of beef to the transfer beef, the concentrations of Pb, Ba and Sb within the larvae decreased to concentrations that were not significantly different from those detected within the larvae feeding on the control piece of beef. Although data was not obtained for larvae that had been feeding for 12, 24 and 36 h, it appears that the larvae are able to eliminate Pb, Ba and Sb within 12 h of transfer from the shot piece of beef. Given these results it is likely, but not measured, that the majority of the Pb, Ba and Sb ingested by the larvae is held in either the crop or midgut of the larvae then later egested and not incorporated within the larval tissues.

The concentrations of Pb and Ba detected in the post-feeding larvae and pupae, which had been feeding on the pieces of beef containing GSR, were significantly higher than the concentrations detected within post-feeding larvae and pupae removed from the control piece of beef. However, Sb concentrations were not significantly different between post-feeding larvae and pupae removed from the pieces of beef containing GSR and post-feeding larvae and pupae removed from the control piece of beef. A large degree of variation was observed within the five replicate post-feeding larvae and pupae removed from the pieces of beef containing GSR. This is probably due to the fact that

once the larvae have stopped actively feeding on the piece of beef containing the GSR, it is impossible to sample larvae that had all been feeding in the same area. It is possible that some of the larvae had been feeding close to the entrance wound in areas containing many GSR particles, whereas others may have been feeding in areas containing little or no GSR particles.

Concentrations of Pb, Ba and Sb detected in the empty puparia of the larvae feeding on the pieces of beef containing GSR were not significantly different from the concentrations detected within puparia from the control piece of beef. The background concentrations of the elements detected in the puparia from the control piece of beef were considerably higher than those observed within the actively feeding larvae on the control piece of beef. This can in part be attributed to the relatively low weight of the empty puparia which can result not only in inaccurate recordings of weight on a four decimal place balance, but also produces a relatively high dilution factor which is undesirable when analysing samples for ultra-trace element concentrations.

The findings of this study suggest that blowfly larvae do ingest gunshot residues when feeding on a piece of beef containing GSR. However, correct interpretation of the results is imperative in establishing an accurate conclusion. The mere presence of Pb, Ba and Sb within blowfly larvae, while extremely indicative of the presence of GSR, is not conclusive evidence for the presence of GSR. In field trials it may be necessary to analyse body tissue (e.g. muscle, bone marrow) of the deceased, from a region far from the suspected wound, for comparison with Pb, Ba and Sb levels within the larvae. In this study, the fact that significantly high concentrations of all three elements were detected within larvae feeding on the shot substrates is certainly extremely indicative of the presence of gunshot residues within the larvae. However, it is judicial at this stage in the absence of field trials as to whether these three elements, within larvae removed from a corpse, could be the result of environmental concentrations (where the corpse was found) or occupational concentrations (what elements the deceased was exposed to in their life) rather than a gunshot incident.

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