Correspondence

Commentary on Goff ML, Miller ML, Paulson JD, Lord WD, Richards E, Omori AI. Effects of 3,4-methelenedioxymethamphetamine in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and detection of the drug in postmortem blood, liver tissue, larvae and pupae. J Forensic Sci 1997;42(2):276–280.

Sir:

The recent paper by Goff et al. (1) demonstrates the effects of drugs and toxins present in decomposing bodies on insect development rates and also how insects and larvae may serve as alternate specimens for toxicological analyses. The experiments involved rearing larvae of *Parasarcophaga ruficornis* on tissues from rabbits previously injected with 3,4-methelenedioxymethamphetamine (MDMA). We have performed similar experiments by rearing *Calliphora vicina* larvae on an artificial food medium spiked with various concentrations of many forensically important drugs and analysing larvae and pupae by HPLC. These studies have shown that larval drug concentrations vary considerably throughout larval development. The pattern seen with amitriptyline, temazepam, trimipramine and trazodone is a gradual rise in larval drug concentration to a peak at about 7–8 days which then diminishes to zero by pupation at 16 days (2, 3). These drugs were undetectable in pupae using routine toxicological techniques. If the same is true of *Parasarcophaga ruficornis,* then the analysis of larvae at day 3 and pupae at day 22 or later (1) would have missed the peak in larval drug concentration. The fact that larvae are able to eliminate drugs with varying levels of efficiency has considerable practical significance since the precipitous decrease in drug concentrations seen in non-feeding larvae and at pupariation make it desirable to sample for toxicological analysis only larvae actively feeding on a corpse (3). Also, some drugs (e.g., acetaminophen and acetylsalicylic acid) are so rapidly eliminated that their absence from larvae does not necessarily indicate that they were also absent from the larval food source (4, 5).

In Goff's experiments there is an apparent quantitative relationship between the MDMA concentration in the food source and that in larvae, although the number of larval rearings is not stated. However, this data should be interpreted with caution since experiments involving rearing large numbers of larvae on the same amitriptyline-laden food source have shown 30-fold variations in larval drug concentration and 4-fold variations in mean larval weight (6). The magnitude of this biological variation renders scientifically unsound any practical attempt to extrapolate the drug concentration in a corpse from that measured in the larvae. Most other workers have found no correlation between the drug concentration in the larvae and in the tissues on which the larvae were feeding (7).

Goff's results show drug concentrations in larvae which are higher than those in the liver on which the larvae were fed. This raises the possibility that bioaccumulation of MDMA in *Parasarcophaga ruficornis* larvae may occur, similar to the situation with mercury in *Calliphoridae* (8). In contrast, our studies, which involve many different antidepressants, analgesics, barbiturates, benzodiazepines and drugs of abuse in *Calliphora vicina* larvae, have shown no such evidence of bioaccumulation, with larval drug concentrations typically being 10–20% of those present in the foodstuff. The highest relative drug concentration (41%) was found with amphetamine sulphate (4). The higher drug levels detected by Goff in larvae and pupae may result from surface contamination by the drug-rich putrefactive residue which accumulates in the rearing medium over time. We have found that drug concentrations in larval and pupal samples which were left unwashed prior to analysis were significantly higher than in adequately washed samples (6). The practical significance of this is that unwashed samples offer the best prospect of success when qualitative detection of drugs in larvae is the objective and that adequate washing of larval samples is required before any quantitative assumptions can be made.

Some of the differences between our results and those of Goff might be explained by the different sample preparation and analytical techniques employed. We found that levels of amitriptyline in larvae and pupae as measured by GC-MS where higher than those measured by HPLC (6). It may be that the more vigorous drug extraction technique used prior to analysis by GC-MS recovers drug which is physically incorporated into the protein matrix of the pupa case and that the less robust technique used prior to analysis by HPLC recovers only the drug present in the soft tissues. Further investigation is required to determine exactly where within the larvae and pupae the drugs accumulate.

Goff found evidence of conversion of the parent drug, MDMA to its metabolite, MDA. This conversion may have taken place either in the food source prior to ingestion by larvae or by metabolism within the larvae. Similarly, we have found evidence of conversion of amitriptyline to nortriptyline (6). It is of interest that after feeding on amitriptyline spiked foodstuff, the ratio of amitriptyline to nortriptyline in larvae was 5:1, which is similar to the ratio seen in the blood and tissues of acute human poisonings.

These findings have important implications for the practice of forensic entomotoxicology (9). Firstly, although migratory and post feeding larvae provide vital information about post mortem interval, only larvae actively feeding on a corpse should be sampled for toxicological analysis. Secondly, although larvae are useful as qualitative toxicological specimens, they appear to be of limited quantitative value. Finally, the absence of drug from larvae does not necessarily imply its absence from the larval food source.

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Authors' Response

Sir:

The recent letter by Sadler et al. reiterates several important points concerning the use of insects as alternative toxicological specimens. We certainly agree that carrion feeding insects provide a potentially valuable source of information and that the current state of research does not allow for accurate quantitative assessments. Our investigations to date, however, have demonstrated the possibility of quantitative correlations between drug concentrations found in mammalian soft tissue and developing insect larvae, chitinous remnants (puparial cases and cast larval skins) and insect fecal material (1, 2). The ability to make quantitative assessments will be dependent on rearing, extraction, and detection methodologies. We have chosen to rear insects on animal models dosed by IV infusion to ensure realistic simulation of decomposition conditions, uniform larval nutrition and development. Our extraction and detection methodologies are based on lengthy research experience on the identification of drugs from nonconventional tissues (hair and nails). The analysis of samples with mass spectrometric techniques has allowed us to identify trace levels of drugs with increased accuracy.

While we agree that fully developed fly larvae currently represent the best source of drug residues, our research has demonstrated the potential toxicological value of developing larvae of other ages and the chitinous by-products of insect development. Sampling of all available immature life stages as well as chitinous remnants and insect fecal material may prove useful. To date only very limited research has been conducted which focusses on the comparison of quantitative drug concentrations in developing insects and their hosts. Our research, while preliminary, points to the potential usefulness of such comparisons in determining acute versus chronic exposure of the host via the ratio of parent compound to metabolite. Little is currently known however about the physiological metabolism of drugs within the developing insects. Our latest work with MDMA suggests a slow larval metabolism of parent drug to normal human metabolites within insects. It should be noted our observations in this regard are limited and somewhat speculative.

Our results with MDMA demonstrate higher levels of drug in larvae than in the liver on which they were fed. We consider this to be a clear indication of bioaccumulation. The process of bioaccumulation is common in a wide variety of insects and has been demonstrated previously in carrion frequenting flies. We discount the notion of surface contamination as our specimens were thoroughly washed prior to analysis. Our research and the work of others currently provides only a glimpse of the potential of entomotoxicology to the forensic sciences. We hope our efforts continue to stimulate research and open discourse in this emerging field.

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Coroner and Medical Examiner Systems

Sir:

In his letter to the editor ("Is The Power of Inquest a Valuable Asset?", JFS, July, 1997), Dr. Don Harper Mills has raised an interesting question which had been discussed in some detail in a joint session conducted at the AAFS 1997 Annual Meeting, "Coroner v. Medical Examiner". In that session, I discussed several inherent advantages of a strong, traditional coroner system, namely, the opportunity to conduct inquests whenever necessary and desirable.

In Allegheny County, all defendants in homicide cases are arraigned at our office, and preliminary hearings are conducted at this facility. We have the power of subpoena and utilize it whenever appropriate to obtain medical records and other pertinent documents, and to ensure the presence of witnesses at the inquests.

We also conduct open inquests on matters that relate to the public health, safety, and welfare, as well as highly controversial cases (e.g., fatal shooting by a white policeman of a young Afro-American teenager).

The advantages of an appointive medical examiner system, including the prerequisite that the M.E. be a Board-certified forensic pathologist, are obvious and well-known to all of us in the

field. Regrettably, however, very few, if any, medical examiner offices have the power to convene inquests, issue subpoenas, and thereby address matters of vital interest and concern to the public.

The challenge for all of us involved in official governmental medical-legal investigation to consider is this—how can the distinct advantages of both the medical examiner and coroner systems be combined so as to accomplish the greatest benefit for society? The simple and arrogant statement that one or the other system is unquestionably superior, while the other is automatically and inherently inept, is a manifestation of a lack of knowledge and practical experience regarding the two systems.

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Commentary on Simmons GT. Findings in gunshot wounds from tandem projectiles. J Forensic Sci 1997 Jul;42(4):678–81.

Sir:

I suggest that a modification is needed in the title of the above-referenced article to indicate that the article deals only with cases in which such tandem projectiles had been produced accidentally. Overlooked in this article was the fact that there have been a lot more tandem projectiles produced on purpose than by accident. There was at least one tandem-projectile handgun cartridge commercially available up until 1994: the Remington 38 Special "Multi-Ball" loading. Two 000 Buckshot (each weighing about 70 grains) were loaded in each of these cartridges. Tandem projectiles have also been used experimentally and developmentally by manufacturers, and by handloaders, for many years. One of the candidates in the Army's competition for the Advanced Combat Rifle, in the 1980s, was, in fact, a tandem cartridge. That was the Colt ACR duplex round in which one 35 and one 31 grain pointed rifle bullets were loaded in a single .223 Remington case.

In 1991, while working at the Wound Ballistics Laboratory at the Letterman Army Institute of Research, I received a call from a medical examiner who told me of an autopsy he had done recently. The death had been caused by a shot from a Remington "Multi-Ball" round. He had expected that the reason for a "Multi-Ball" loading was to increase the probability of a hit—but was surprised that in his case the balls had both entered through a single entrance wound—negating such a purpose. The wound could not be differentiated from one caused by a single projectile, unlike the wound shown as Fig. 2, in the article by Simmons, in which there appears to be a scalloping effect indicating slight separation of the projectiles.

We obtained Remington "Multi-Ball" cartridges and fired five of them into 10% ordnance gelatin, at a distance of 10 feet, from a .357 Magnum chambered Ruger revolver with a 4 inch barrel. To our surprise, in all five shots the two 000 Buckshot remained nested together in the air and entered the gelatin as a single projectile. Even after entering the gelatin tissue simulant, they remained together for distances of 13, 22, 41, 28, and 45 cm before they separated and parted company at an angle between the two paths of about fifteen degrees.

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Authors' Response

Sir:

I thank Dr. Fackler for his interest in my paper published previously in J Forensic Sci 1997;42(4):678–681. The terms "tandem projectiles" or "tandem bullets" in all of the medical forensic literature I reviewed were used only to refer to those cases where this phenomenon had been accidently produced (1–3). However, as Dr. Fackler points out, deliberately manufactured multiple bullet loadings exist and are possible sources of tandem projectiles. I therefore appreciate his suggestion that when using the term "tandem projectiles" it would be appropriate to specify whether one is referring to accidently and/or deliberately produced tandem projectiles.

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Commentary on Sadler DW, Robertson L, Brown G, Fuke C, Pounder DJ. Barbiturates and analgesics in *Calliphora vicina larvae.* J Forensic Sci 1997;42(3):481–485.

Sir:

Recent work in the field of forensic entomotoxicology has shown the undoubted value of using larvae found actively feeding on a drug-laden corpse as alternative toxicological specimens (1–7). However, our recent paper highlights some of the practical limitations of using this approach (1). Although the barbiturates thiopentone, phenobarbitone, amylobarbitone, barbitone and brallobarbitone all share the same basic chemical structure, which is based on a pyrimidine ring, differences in their side chain structure have a major impact on the way in which the drugs are metabolized by larvae. Specifically, whereas barbitone accumulated significantly in *Calliphora vicina* larvae, amylobarbitone and phenobarbitone showed much lesser accumulation, and thiopentone was not detectable. Both thiopentone and phenobarbitone became lethal to larvae at higher concentrations and marked variability in larval size and mortality was also noted in larvae fed on the other barbiturates.

Using the same experimental design *Calliphora vicina* larvae were reared on artificial foodstuffs containing various concentrations of four common benzodiazepines. Larval drug concentrations in day seven larvae, measured by high performance liquid chromatography, are shown in Table 1. Larvae accumulate flurazepam to only a minor degree when the drug is present in the foodstuff at low concentrations but appear to successfully eliminate higher concentrations of the drug, possibly via inducible excretory mechanisms. Loprazolam is similarly rapidly eliminated by larvae, so that it is not detectable. By contrast, both bromazepam and diazepam are detectable but the relationship between larval drug concentration and foodstuff concentration differs. Thus, the benzodiazepine group of drugs, as well as the barbiturate group, show unpredictable patterns of drug accumulation in larvae. This reinforces the view

TABLE 1—*Bromazepam, Diazepam, Flurazepam and Loprazolam in Day Seven Larvae.*

Mean (Range) Drug Conc. in Foodstuff (mmol/g) n=5		Mean (Range) Larval Weight (mg)	Mean (Range) Larval Drug Conc (mmol/g)	Larva: Foodstuff Drug Ratio
Bromazepam	42 (40–45)	25 (16–34)	9.3 (0–10.2) n=3	22%
	78 (74–83)	44 (27–54)	38.7 (24.4–50.7) n=4	49%
Diazepam	17 (5–32)	32 (19–55)	2.9 (0.3–6.8) n=3	17%
	108 (87–120)	36 (25–42)	1.3 (0.2–2.4) n=3	1.2%
Flurazepam	64 (51–78)	56 (34–84)	53 (0–13.3) n=4	8.3%
	102 (93–112)	71 (56–78)	0 n=3	0%
	180 (158–213)	50 (48–52)	0 n=3	0%
Loprazolam	28 (21–47)	42 (33–58)	0 n=3	0%
	85 (79–109)	29 (21–40)	0 n=3	0%

that it is impossible to predict, on the basis of chemical structure, which drugs are likely to be detectable in *Calliphora vicina* larvae. Also, when a drug is detectable in larvae, extrapolation from that concentration to the concentration in the food source—in practice, a corpse—is difficult if not impossible in the absence of test study data.

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