*Valéry Hédouin,[1] M.D., Ph.D.; Benoit Bourel,[1,2] Ph.D.; Anne Bécart,[1] D.D.S.; Gilles Tournel,[1] M.D.; Marc Deveaux,[1] Pharm.D., Ph.D.; M. Lee Goff,[3] Ph.D.; Didier Gosset,[1] M.D., Ph.D.*

# Determination of Drug Levels in Larvae of *Protophormia terraenovae* and *Calliphora vicina* (Diptera: Calliphoridae) Reared on Rabbit Carcasses Containing Morphine

**REFERENCE:** Hédouin V, Bourel B, Bécart A, Tournel G, Deveaux M, Goff ML, Gosset D. Determination of drug levels in larvae of *Protophormia terraenovae* and *Calliphora vicina* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. J Forensic Sci 2001;46(1):12–14.

**ABSTRACT:** Two species of blow flies (Diptera: Calliphoridae) were reared on tissues from rabbits administered different dosages of morphine. These species, *Protophormia terraenovae* and *Calliphora vicina* are among the first wave of insects colonizing a dead body. Two series of 3 rabbits were given dosages of 10, 20, and 40 mg/h of morphine over a 3 h period via ear artery perfusion. A morphine blood level plateau was attained after 1 h of perfusion. Two other rabbits were used as controls. Samples of tissues collected from rabbits using a coelioscopic technique were determined to have morphine concentrations similar to those encountered in human overdoses and were correlated with dosages of morphine administered. All samples from control rabbits were negative for morphine. Larvae and puparia of both species were regularly collected from each rabbit for toxicological analysis. Concentrations of morphine in larvae reared on rabbit carcasses containing morphine were significantly lower than concentrations found in the tissues. There was a decrease in concentration in morphine observed in transition from feeding 3rd instar larva to puparium. A correlation between larval concentration and tissue concentration was found only in feeding 3rd instar larvae.

**KEYWORDS:** forensic science, morphine, heroin, entomotoxicology, rabbits, forensic entomology

In earlier studies using a controlled experimental animal model, we determined that concentrations of morphine in larvae of *Lucilia sericata* fed on tissues containing morphine were 30 to 100 times lower than in the tissues (1). A correlation was observed between morphine concentrations in larvae and concentrations in muscle and fat tissues only during the feeding portion of the 3rd instar. No correlations were observed for other developmental stages and metabolism of the drug by the larvae appears to vary with stage of development. In the present study, we evaluate the development of two additional species of Calliphoridae under the same experimental conditions and seek to analyze possible variations in the pharmacokinetics for these species. The species, *Protophormia terraenovae* and *Calliphora vicina*, are among the earliest invaders of a decomposing body and are frequently used estimations of the postmortem interval. We have investigated the concentrations of morphine in the feeding 3rd instar larvae, prepuparial (post-feeding) larvae, and puparia for these species reared on rabbit carcasses containing different concentrations of morphine and attempted to detect correlations between concentrations of morphine in tissues used as food and the concentrations in the insects fed on those tissues.

## Materials and Methods

Two series of domestic rabbits with weights ranging from 2.68 to 4.50 kg were administered dosages of 10, 20 and 40 mg, respectively, of morphine hydrochloride via ear artery perfusion over a 3 h period (0.08, 16.16, and 32.32 mg free base morphine equivalent). Morphine hydrochloride was diluted in 150 mL isotonic saline solution for administration. Theses dosages and rates of perfusion were calculated to obtain morphine tissue concentrations similar to those encountered in cases of fatal human overdoses. These parameters were calculated based on earlier studies (2). During the perfusion, a lethal plateau of morphine concentration was obtained after 100 min. This level was consistent with the dosage administered and remained constant for the duration of the perfusion. For each study, one other rabbit, weights of 2.69 and 4.42 kg, respectively, were used as a control and received only 150 mL of isotonic saline via ear artery perfusion.

Following perfusion, rabbits were sacrificed in a Carbon Dioxide Chamber. Samples of organs and tissues (cardiac blood, liver, fat, deep muscle, and skin) were taken as in previous studies (1,2) and analyzed for morphine concentration. Immediately following sampling, tissues were homogenized in a Potter-Elvehjem homogenizer and then centrifuged. Two aliquots were made of the supernatant and these were stored at −20°C until analysis.

Eggs of uniform age of *P. terraenovae* and *C. vicina* were obtained from colonies of these species which had been established from specimens collected from decomposing human remains. Approximately 400 eggs of *P. terraenovae* were placed in the eyes, nose, and mouth of rabbit carcasses labeled as R1, R2, R3, and C1. In a similar manner, approximately 400 eggs of *C. vicina* were placed into the eyes, nose, and mouth of rabbit carcasses labeled as

[1] Institut de Médecine Légale et de Médecine Sociale, Faculté de Médecine, Place Théo Varlet, 59000 Lille, France.
[2] Laboratoire Environnement et Senté, Faculté Libre des Sciences, 55 rue du Port, 59046 Lille Cedex, France.
[3] Department of Entomology, University of Hawaii at Manoa, Honolulu, Hawaii.

Received 25 Jan. 1999; and in revised form 1 Feb. 2000; accepted 4 Feb. 2000.

R4, R5, R6, and C2. Time at which eggs were placed was labeled as TO. Carcasses were placed into plastic boxes covered with wire netting to prevent contamination by other insect species. Boxes were placed in a closed room under normal daylight conditions. Temperatures were recorded during each step in the process. At regular intervals, random samples of 10 larvae were taken from each carcass, dried, and frozen. For analysis, larvae were homogenized in a Potter-Elvehjem homogenizer, centrifuged, and the supernatant analyzed. Morphine concentrations were determined using a sensitive and specific technique (Coat-a-count Serum morphine RIA, Behring Diagnostic, Rueil, France, Detection limit: 1 ng/mL). Samples anticipated to have concentrations greater than the highest concentration (250 ng/mL) were diluted with human serum prior to assay. Following manufacturer's instructions, concentrations were determined twice.

For statistical evaluation, a linear regression was used of morphine in tissues against initial dosage, and concentration in larvae against concentration of drug in muscle, skin, and fat tissues.

## Results

All blood and tissue samples from rabbits receiving dosages of morphine hydrochloride were positive for the drug, while all samples from the controls were negative. For each rabbit, morphine tissue concentrations were consistent with dosage of morphine administered (Table 1); however, no strict proportionality was observed. Morphine concentrations in tissues from R2 were 1.5 to 3.2 times higher than for R1, and those from R3 were 1.3 to 3.0 times higher than for R2. Morphine concentrations for R5 were 1.4 to 2.3 times higher than for R4 and those for R6 were 1.4 to 2.6 times higher than for R5 (Table 1). Development of larvae of *P. terraenovae* was as follows: 3rd instar reached at day 5, prepuparial stage by day 11, and pupariation at day 13. Morphine concentrations for stages are given in Table 2. Development for *C. vicina* was as follows: 3rd instar reached at day 5; prepuparial stage by day 8, and pupariation on day 13. Concentrations of morphine in developmental stages were significantly lower than those in tissues. Regression analysis shows that concentrations in larvae of *P. terraenovae* larvae sampled from days 6 and 11 correlate well with initial dosage of morphine and concentrations in skin, muscle, and fat tissues as a food source (Table 2). Regression analysis shows that larvae of *C. vicina* sampled on days 6 and 7 correlate well with initial dosage of morphine administered as well as morphine concentrations in skin, muscle, and fat tissues used as a food source (Table 3).

Analyses were not performed on larvae collected on day 8, as different stages of development were found simultaneously on the carcasses (late feeding 3rd and prepuparial larvae).

TABLE 1—*Concentration of morphine in rabbits' tissues according to the flow of morphine perfusion (in ng/g).*

| Tissues | 10 mg/h* | 20 mg/h | 40 mg/h | *r* |
|---|---|---|---|---|
| Cardiac blood | 1293† | 2825 | 4056 | 0.99 |
| | 895‡ | 2098 | 2851 | 0.98 |
| Liver | 1572 | 3814 | 6659 | 0.99 |
| | 1857 | 2614 | 4296 | 0.99 |
| Fat | 1676 | 3299 | 5804 | 0.99 |
| | 1917 | 3022 | 4010 | 0.98 |
| Skin | 945 | 1401 | 3205 | 0.99 |
| | 441 | 591 | 1518 | 0.98 |
| Muscle | 800 | 2588 | 7713 | 0.98 |
| | 777 | 1341 | 2294 | 0.99 |

* represents the flow of morphine perfusion.
† represents the concentrations obtained for the rabbits R1, R2, and R3.
‡ represents the concentrations obtained for rabbits R4, R5, and R6.
NOTE: *r* is the correlation coefficient between the injected dosage and the concentrations in the organs.

## Discussion

In cases of advanced decomposition when tissues and body fluids are not available, insects can serve as alternate samples for toxicological analyses. Goff and Lord (3) have extensively discussed the advantages and difficulties in this new area of forensic investigation and several cases and studies have been published (4,5). In these cases, analyses of larvae provided a qualitative assessment of the presence of a drug. In an earlier study, we investigated the concentrations of morphine in each stage in the development of the blow fly *Lucilia sericata* (Diptera: Calliphoridae) reared on tissues from rabbits administered different dosages of morphine (1). That study demonstrated that concentrations in the larvae were 45 to 100 times less than in the tissues used as a food source. Regression analysis demonstrated a correlation between concentrations in the 3rd instar larvae, initial dosage of morphine administered and con-

TABLE 2—*Concentration of morphine in ng/g from larval to pupal stages* (Protophormia terraenovae).

| Time (days) | $T°^{+}$ | *R*1 | *R*2 | *R*3 | $r_1$ | $r_2$ | $r_3$ |
|---|---|---|---|---|---|---|---|
| 5 | 21–24 | 81 (2 + 3) | 90 (2) | 584 (2) | 0.93 | 0.90 | 0.98 |
| 6 | 21–24 | 60 (2 + 3) | 168 (3) | 389 (3) | 0.99 | 0.99 | 0.99 |
| 7 | 20–23 | 58 (3) | 214 (3) | 470 (3) | 0.99 | 0.99 | 0.98 |
| 8 | 19–23 | 55 (3) | 262 (3) | 542 (3) | 0.99 | 0.99 | 0.97 |
| 9 | 21–25 | 35 (3) | 146 (3) | 281 (3) | 0.99 | 0.99 | 0.96 |
| 11 | 21–25 | 14 (pp) | 51 (pp) | 120 (pp) | 0.99 | 0.99 | 0.99 |
| 13 | 20–22 | 5 (p) | 23 (p) | 18 (p) | 0.60 | 0.65 | 0.43 |

NOTE:
(2) larvae stage 2.
(3) larvae stage 3.
(pp) post-feeding stage.
(p) pupal stage.
$r_1$ is the correlation coefficient between the muscle and the larval concentrations.
$r_2$ is the correlation coefficient between the skin and the larval concentrations.
$r_3$ is the correlation coefficient between the fat and the larval concentrations.
$+$ are the minimal and the maximal temperatures exprimed in Celsius degrees.

TABLE 3—*Concentration of morphine in ng/g from larval to pupal stages (*Calliphora vicina*).*

| Time (days) | $T°^{+}$ | R4 | R5 | R6 | $r_1$ | $r_2$ | $r_3$ |
|---|---|---|---|---|---|---|---|
| 5 | 18–24 | 118 (3) | 42 (3) | 161 (3) | 0.75 | 0.82 | 0.84 |
| 6 | 20–23 | 163 (3) | 145 (3) | 420 (3) | 0.90 | 0.95 | 0.99 |
| 7 | 20–22 | 147 (3) | 130 (3) | 425 (3) | 0.88 | 0.94 | 0.99 |
| 8 | 20–29 | 19 (3 + pp) | 74 (3 + pp) | 191 (3 + pp) | – | – | – |
| 9 | 20–22 | 5 (pp) | 1 (pp) | 2 (pp) | 0.36 | 0.38 | 0.32 |
| 13 | 18–27 | 0 (p) | 0 (p) | 3 (p) | 0.690 | 0.79 | 0.92 |

NOTE:
(2) larvae stage 2.
(3) larvae stage 3.
(pp) post-feeding stage.
(p) pupal stage.
$r_1$ is the correlation coefficient between the muscle and the larval concentrations.
$r_2$ is the correlation coefficient between the skin and the larval concentrations.
$r_3$ is the correlation coefficient between the fat and the larval concentrations.
– not done.
+ are the minimal and the maximal temperatures exprimed in Celsius degrees.

centrations in muscle and fat tissues used as a food source. In the present study, we have investigated two additional species of calliphorids within the initial wave on colonizers of a body. In order to compare results, the same animal model was used and preparations of animals, tissues and organs were similar (1). This model also allows for a controlled level of the drug in blood and other tissues as well as an approximation of visceral levels of morphine encountered in fatal human overdoses (6).

There was a good correlation between concentration of morphine administered and tissues concentrations. For each organ sampled, morphine level detected was within the range of normal human lethal concentrations (Table 1). All tissues sampled showed a good correlation with administered dosage, particularly the muscle, skin, and fat, which are normally the first tissues consumed by larvae feeding on a body. A good correlation was also observed between administered dosage and cardiac blood and liver tissue. Morphine concentrations in larvae of *P. terraenovae* and *C. vicina* were 30 to 100 times lower than in the tissues used as a food source, similar to results obtained earlier for *L. sericata* (1). Theses results differ from those obtained by Introna et al. (7). The concentrations in larvae were quite similar to those in the human tissues used. While both studies used the same analytical technique (RIA), same fly species (*C. vicina*), and sample preparation, one difference did exist. In their study, Introna et al. (7) used human liver tissue as a rearing medium, while we used entire rabbit carcasses. Differences in concentrations in larvae due to differences in feeding substrates are not known. The RIA assay process used in the present study measures only free morphine (8) and the metabolism of morphine in insects is unknown at present. As with *L. sericata*, morphine concentrations in 3rd instar larvae of *P. terranovae* and *C. vicina* show a significant correlation with concentrations found in muscle, skin, and fat tissues (Tables 2 & 3). This correlation is not seen in later stages of development. The late feeding 3rd instar period of development for all 3 species is a period of rapid feeding prior to the start of the post-feeding prepuparial stage. During this period, unlike other stages, the rate of absorption equals the rate of elimination of the drug. The results obtained in these studies do not allow for extrapolation to human cases but confirm that detection of morphine is most easily accomplished by analyses of 3rd instar larvae. A small amount of morphine is still measurable in the prepuparial and puparial stages, as has been noted in studies dealing with other drugs (9). The larval metabolism most probably plays a determinant role in the level of the drug present (10–12). Concentrations of morphine present in earlier stages of development have not been presented here as they were variable. Unlike the 3rd instar larvae, water must be added to the much smaller 1st and 2nd instar larvae before processing and this dilution has presented difficulties in interpretation of results.

## References


1. Hédouin V, Bourel B, Martin-Bouyer L, Bécart A, Tournel G, Deveaux M, et al. Determination of drug levels in larvae of *Lucilia sericata* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. J Forensic Sci 1999;44(2):351–3.
2. Hédouin V, Bourel B, Martin-Bouyer L, Bécart A, Tournel G, Deveaux M, et al. Morphine perfused rabbits: a tool for experiments in forensic entomotoxicology. J Forensic Sci 1999;44(2):347–50.
3. Goff ML, Lord WD. Entomotoxicology: a new area for forensic investigation. Amer J Forensic Med Pathol 1994;15:51–7.
4. Gunatilake K, Goff ML. Detection of organophosphate poisoning in a putrefying body by analyzing arthropod larvae. J Forensic Sci 1989;34:714–6.
5. Beyer JC, Enos WF, Stajic M. Drug identification through analysis of maggots. J Forensic Sci 1980;25:411–2.
6. Baselt RC, Cravey RH, editors. Disposition of toxic drugs and chemicals in man. Foster City: Chemical Toxicology Institute, 1995.
7. Introna F, LoDico C, Caplan YH, Smialek JE. Opiate analysis in cadaveric blowfly larvae as an indicator of narcotic intoxication. J Forensic Sci 1990;35:118–22.
8. Spiehler V, Brown R. Unconjugated morphine in blood by radioimmunoassay and gas chromatography/mass spectrometry. J Forensic Sci 1987;32:906–16.
9. Sadler DW, Court FG, Fuke C, Pounder DJ. Drug accumulation and elimination in *Calliphora vicina* larvae. Forensic Sci Int 1995;71:191–7.
10. Kintz P, Tracqui A, Mangin P. Analysis of opiates in fly larvae sampled on a putrefied cadaver. J Forensic Sci 1994;34:95–7.
11. Goff ML, Brown WA, Hewadikaram KA, Omori AI. Effect of heroin in decomposing tissues on the development rate of *Boettcherisca peregrina* (Diptera: Sarcophagidae) and implications of those effect on estimation of postmortem intervals using arthropod development patterns. J Forensic Sci 1991;36:537–42.
12. Wilson Z, Hubbard S, Pounder DJ. Drug analysis in fly larvae. Am J Forensic Med Pathol 1993;14:118–20.



Additional information information and reprint requests:
Professor Didier Gosset
Institut de Medecine Legale et de Medecine Sociale
Place Theo Varlet
Lille 59 000
France

# ERRATA/CORRECTIONS

We have identified a number of instances in which the authors of work published in the Journal of Forensic Sciences have miscited papers originally published in the Journal of the Forensic Science Society as having been published in the Journal of Forensic Sciences.

The known instances of this error for volume 44 of the Journal of Forensic Sciences are detailed/corrected below. We have not checked other volumes for similar errors. The Journal of Forensic Sciences regrets these errors.

Since 1995 (Volume 35), the Journal of the Forensic Science Society has been published under the title "Science and Justice."

The editors of both journals take this opportunity to remind authors of the necessity for ensuring the accuracy of the references they cite in manuscripts submitted for publication. The Instructions for Authors of both journals make it clear that accuracy of reference citation is the responsibility of authors, and good scholarship demands attention to this matter.

| | |
|---|---|
| A. R. W. Forrest | R. E. Gaensslen |
| Editor, Science and Justice | Editor, Journal of Forensic Sciences |

The journal citation in reference 7 in Foreman LA, Smith AFM, Evett IW. Bayesian validation of a quadriplex STR profiling system for identification purposes. should read: J Forensic Sci Soc 1992;32:5–14.

The journal citation in reference 5 in Bourel B, Hedouin V, Martin-Bouyer L, Becart A, Tournel G, Deveaux M, Gosset D. Effects of morphine in decomposing bodies on the development of Lucila sericata (Diptera: Calliphoridae). should read: J Forensic Sci Soc 1991;31:469–72.

The journal citation in reference 8 in Hedouin V, Bourel B, Martin-Bouyer L, Becart A, Tournel G, Deveaux M, Gosset D. Determination of drug levels in larvae of Lucila sericata (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. should read: J Forensic Sci Soc 1994;34:95–7.

The journal citation in reference 15 in Hedouin V, Bourel B, Martin-Bouyer L, Becart A, Tournel G, Deveaux M, Gosset D. Morphine perfused rabbits: A tool for experiments in forensic entomotoxicology. should read: J Forensic Sci Soc 1991;31: 469–72.

The journal citation in reference 10 in McDermott SD, Willis SM, McCullough JP. The evidential value of paint. Part II. A Bayesian approach. should read: J Forensic Sci Soc 1992;32: 333–48.

The journal citations in references 4 and 5 in Infante F, Dominguez E, Trujillo D, Luna A. Metal contamination in illicit samples of heroin. should read for 4: J Forensic Sci Soc 1979;19:203–9. and for 5: J Forensic Sci Soc 1980;20:177–81. [in reference 5 only the volume number is miscited]. And in both references, the lead author's name is "Joyce JR."

The journal citation in reference 1 in Savolainen P, Lundeberg J. Forensic evidence based on mtDNA from dog and wolf hairs. should read: J Forensic Sci Soc 1988;28:335–9.

The journal citation in reference 1 in Kupfer DM, Chaturvedi AK, Canfield DV, Roe BA. PCR-based identification of postmortem microbial contaminants—A preliminary study. should read: J Forensic Sci Soc 1968;8:73–6.

In every instance cited above, future citations of the J Forensic Sci papers containing the errors should contain the following: [published erratum appears in J Forensic Sci 2001 Jan;46(1)] immediately following the article title and before the journal citation, in accordance with the Uniform Requirements for the Submission of Manuscripts to Biomedical Journals style.