# CASE REPORT

*Orietta Crippa,*[1] *B.Sc.; Aldo Polettini,*[1] *B.Sc.; and Francesco M. Avato,*[2] *M.D.*

# Lethal Poisoning by Zipeprol in Drug Addicts

**REFERENCE:** Crippa, O., Polettini, A., and Avato, F. M., **"Lethal Poisoning by Zipeprol in Drug Addicts,"** *Journal of Forensic Sciences*, JFSCA, Vol. 35, No. 4, July 1990, pp. 992–999.

**ABSTRACT:** Two cases of lethal intoxication involving or due to oral ingestion of zipeprol are described. The two cases concerned abusers of the substance for nonmedical purposes. Data regarding the distribution of the unmodified drug in biological fluids and tissues are presented.

**KEYWORDS:** toxicology, poisoning, abuse drugs, zipeprol

Zipeprol [1-(2-methoxy-2-phenylethyl)-4-(2-hydroxy-3-methoxy-3-phenylpropyl)piperazine] hydrochloride is an antitussive possessing bronchospasmolytic and mucolytic activity, as well, and lacking opiate-like side effects. Because of its molecular structure, it is a mild antihistamine and shows anticholinergic effects. Its clinical effectiveness has been proved and, in animals, it does not have side effects on the cardiovascular system, intestinal functions, or central nervous system, unlike opiate antitussives [*1,2*]. The pharmacokinetics and metabolism of zipeprol in man and animals are known. The substance is well absorbed after oral administration: the peak plasma level is attained 15 min later and the excretion peak of the unchanged drug and its metabolites occurs about 1 h after ingestion.

The fraction of the dose excreted unmodified in urine is 1 to 5%. Two main *N*-dealkylated metabolites [1-(2-methoxy-2-phenylethyl)piperazine and 1-(2-hydroxy-3-methoxy-3-phenylpropyl)piperazine] were identified, and these two account for 4 to 13% and 14 to 25% of the dose, respectively. Additional metabolites were also detected in human urine [*3–6*].

Zipeprol is commercially available in Italy in different pharmaceutical dosage forms (coated tablets: 75 mg; syrup: 0.3 and 0.5%; suppositories: 50 and 150 mg). The recommended daily dose is 150 to 350 mg in adults and 3 to 5 mg/kg in children under 14 years of age.

Received for publication 6 April 1989; revised manuscript received 18 July 1989; accepted for publication 20 July 1989.

[1]Researcher and postgraduate student, respectively, Institute of Legal Medicine, University of Pavia, Italy.

[2]Associate professor, Institute of Legal Medicine, University of Ferrara, Italy.

The present report concerns two lethal cases of acute oral poisoning involving zipeprol, and specific reference is made to the analytical point of view.

## Case Histories

*Case No. 1*

A 22-year-old drug addict was admitted to a hospital in a presumed opioid coma. After intravenous naloxone and oxygen therapy, he recovered and ½ h after admission he left the hospital. About 3 h later, he was brought back lifeless. Needle marks, some of them fresh, were visible on his arms. The autopsy and histology results revealed congestion (in the brain, lungs, kidneys and liver), microhemorrhages (in the heart, spleen, and lungs), edema (in the lungs), and myocardium fragmentation.

*Case No. 2*

A 22-year-old drug addict was found dead on the road. Fresh needle marks were not present, nor were syringes found near the body. The autopsy and histology results revealed thymic hyperplasia, massive and widespread congestion (in the hypophysis, brain, lungs, liver, kidneys, and suprarenal glands), microhemorrhages (in the heart, spleen, lungs, and thymus), edema (in the brain, kidneys, and lungs), and myocardium dissociation and fragmentation.

## Toxicological Analyses

Initially, the examinations were performed on the biological fluids to test for the presence of the following substances: opiates and cocaine by radioimmunoassay (Abuscreen Roche); methadone, amphetamine, barbiturates, phenothiazines, tricyclic antidepressants, pyrazolone analgesics and local anesthetics by gas chromatography (GC) with nitrogen/phosphorous detector (NPD); benzodiazepines by GC with electron capture detector; ethyl alcohol by GC with head space sampling and flame ionization detector. Organic nonvolatile compounds were searched for in tissue homogenates and gastric contents, which were extracted using the Stas-Otto method, according to Clarke [7], and the extracts were examined by thin-layer chromatography (TLC). These analyses showed that zipeprol was present in both subjects. In Case No. 1, phendimetrazine and its active metabolite phenmetrazine were also found in tissues and fluids, in addition to low levels of opiates in urine and bile. No other drugs beside zipeprol were found in Case No. 2.

The TLC behavior of zipeprol is shown in Table 1.

In Case No. 2, the identification of zipeprol was confirmed by GC/mass spectrometry (GC/MS). The chromatogram of a blood extract and the mass spectra of the zipeprol peak (see *arrow*) in the sample and that of the zipeprol standard are shown in Figs. 1 and 2.

In this paper only the analytical methods performed to quantitate zipeprol are reported.

## Methods

*Case No. 1*

Samples of biological fluids (1 mL) and of tissues and gastric contents (1 g) (the tissues and gastric contents having been homogenized with water at a 1:1 ratio) were spiked with a suitable quantity of internal standard (clotiazam), alkalinized with a phosphate buffer (pH 12), and extracted by vortexing with diethyl ether. The organic phase was

TABLE 1*a*—*Results of thin-layer chromatography of zipeprol.*

| Migration Solvent | Absolute Rf, ×100 |
|---|---|
| Toluene/acetone/ethanol/$NH_4OH$ 30% (45:45:7:3) | 63 |
| Ethyl acetate/methanol/$NH_4OH$ 30% (85:10:10) | 90 |
| Benzene/acetone/petroleum ether/$NH_4OH$ (35:35:35:1) | 45 |
| Methanol/$NH_4OH$ (100:1.5) | 90 |
| Chloroform/acetone (70:30) | 8 |

TABLE 1*b*—*Reactivity results for zipeprol.*

| Spray | Color |
|---|---|
| Marquis | yellow |
| Dragendorff | orange |
| Acidified iodoplatinate | purple |
| Potassium permanganate | yellow |

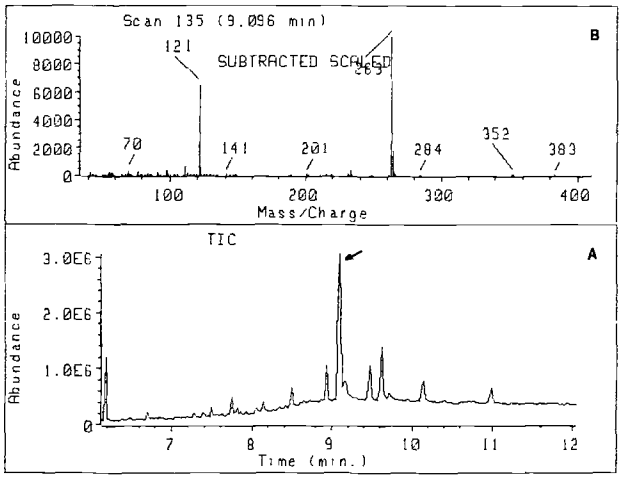


FIG. 1—(a) *Total ion chromatogram of a blood extract (Case No. 2);* (b) *mass spectrum of the peak indicated by the arrow (zipeprol).*

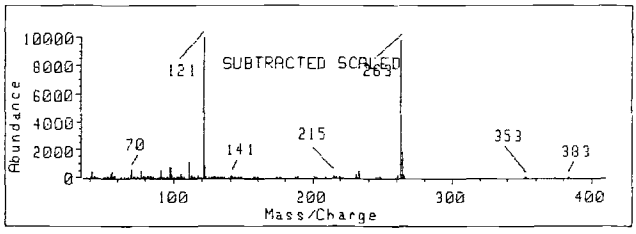


FIG. 2—*Mass spectrum of the zipeprol standard.*

separated and extracted with 4*N* hydrochloric acid (HCl), and the acid phase was alkalinized with 40% sodium hydroxide (NaOH) and back-extracted with diethyl ether. The organic extract was evaporated to dryness under nitrogen, and the residue was dissolved in methanol and examined under the following instrumental conditions:

| | |
|---|---|
| Instrument | gas chromatograph, Perkin Elmer Model Sigma 3 B |
| Column | OV-17 3% on GCQ 100 to 120 mesh [1-m by 2-mm inside diameter (ID) glass column] |
| Temperature | 260°C in the oven; 280°C at the injection port |
| Detector | NPD (390°C) |
| Carrier gas | $N_2$ (flow rate, 30 mL/min) |

*Case No. 2*

Samples of biological fluids (0.25 to 0.5 mL) and samples of tissues and gastric contents (homogenized with water at a 1:1 ratio) were spiked with a suitable quantity of internal standard (eprazinone), alkalinized with borate buffer (pH 9) and vortexed with a benzene/methylene chloride mix (9:1). The organic phase was separated and extracted with 1*N* sulfuric acid ($H_2SO_4$), and the acid phase was neutralized with sodium bicarbonate ($NaHCO_3$), alkalinized with a pH 9 borate buffer, and back-extracted with the above-mentioned solvent mixture. The organic phase was dried under nitrogen and the residue, reconstituted in methanol, was examined under the following instrumental conditions:

| | |
|---|---|
| Instrument | Hewlett-Packard 5890 A gas chromatograph equipped with a Hewlett-Packard mass selective detector, Model 5970 |
| Column | Hewlett-Packard capillary SE-30 (12.5-m by 0.20-mm ID); film thickness, 0.33 μm |
| Column temperature | programed from 50°C (0.5-min initial isotherm) to 80°C at 70°/min; from 80 to 180°C at 40°/min; and from 180 to 290°C at 27°C/min; 5-min final isotherm |
| Injection port temperature | (on column) 50°C |
| Interface temperature | 280°C |
| Detection | selected ion monitoring for the following ions: 263 and 264 atomic mass units (amu) (for zipeprol) and 245 and 246 amu (for eprazinone) |
| Carrier gas | helium (flow rate, 0.5 mL/min) |

The dosage was performed with reference to recovery trials from "blank" tissues and fluids to which zipeprol had been added in quantities that varied according to estimates formulated in preliminary analyses of samples. Internal standards were added in varying and proportional quantities, according to the roughly estimated amounts of zipeprol, in order to obtain comparable peak areas or heights (zipeprol/clotiazam ratio, 3:1; zipeprol/eprazinone ratio, 5:4).

Each sample received the same quantity of internal standard as that used in the corresponding dosage trials.

## Results

The concentrations of zipeprol and other drugs found in tissues and biological fluids in the two cases studied are listed in Table 2. Figure 3 shows (*a*) a gas chromatogram of "blank" blood spiked with 5 μg/mL zipeprol and 4 μg/mL internal standard; (*b*) a gas chromatogram of blood from Case No. 2 (internal standard, 4 μg/mL); and (*c*) ion chromatograms at 245 amu (internal standard) and 263 amu (zipeprol) of blood from Case No. 2.

TABLE 2—*Drug concentrations in the two cases.*[a]

| Sample | Case No. 1 | | | | Case No. 2, |
|---|---|---|---|---|---|
| | Phendimetrazine | Phenmetrazine | Opiates | Zipeprol | Zipeprol |
| Brain | 1.3 μ/g | 3.7 μg/g | NP | 7.7 μg/g | 15.8 μg/g |
| Lungs | 2.4 μg/g | 7.2 μg/g | NP | 25.6 μg/g | 35.5 μg/g |
| Liver | 1.6 μg/g | 2.8 μg/g | NP | 13.2 μg/g | 38.3 μg/g |
| Kidney | 3.1 μg/g | 3.0 μg/g | NP | 18.3 μg/g | 21.4 μg/g |
| Blood | 1.3 μg/mL | 2.0 μg/mL | ND | 10.6 μg/mL | 5.8 μg/mL |
| Bile | 4.8 μg/mL | 7.9 μg/mL | 1.80 μg/mL | 24.9 μg/mL | 20.1 μg/mL |
| Urine | 17.7 μg/mL | 28.9 μg/mL | 0.27 μg/mL | 170.0 μg/mL | NA |
| Gastric contents | 6.0 μg/g | 5.2 μg/g | NP | 81.5 μg/g | 60.4 μg/g |

[a]Abbreviations: NP = analysis not performed; NA = not available; ND = not detected (<0.02 μg/mL)

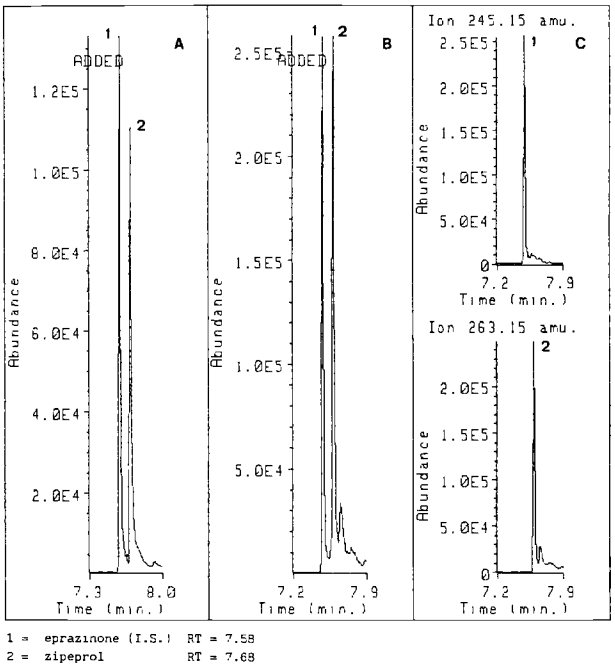


FIG. 3—(a) *Gas chromatogram of "blank" blood spiked with 5 μg/mL zipeprol and 4 μg/mL internal standard;* (b) *gas chromatogram of blood from Case No. 2 (internal standard = 4 μg/mL);* (c) *ion chromatograms at 245 amu (internal standard) and 263 amu (zipeprol) of blood from Case No. 2.*

## Discussion

The abuse of zipeprol by drug addicts has been frequently observed in Italy. Moroni and colleagues [*8*] have indicated 32 cases of acute intoxication due to zipeprol, of which 29 concerned zipeprol abuse by young people (average age, 19 years). The dose taken for recreational purposes varied from 11 to 28 mg/kg. The most obvious toxic consequences were severe neurological troubles (convulsions and coma).

Perraro and Beorchia [*9*] refer to two zipeprol abusers who suffered repeatedly from toxic syndromes associated with doses in the range of 750 to 975 mg. One of these abusers also had a cerebral edema with convulsions. Abusers all describe zipeprol effects as hallucinatory and producing a sense of euphoria, which diminishes rapidly about 1.5 to 2 h after ingestion. The psychodyslectic phase of intoxication is followed by a depressive rebound associated with retrograde amnesia. The psychotoxic syndrome manifests itself after oral ingestion of doses not less than 300 mg; some abusers take zipeprol rectally, using even higher doses (up to 2250 mg or more) [*10*].

Fatalities, however, are relatively infrequent. The pathogenesis, on the basis of the clinically observed overdosage syndrome, is a neurotoxic phenomenon, a well-known characteristic of piperazinic compounds [*11,12*].

No lethal intoxication due to zipeprol has been reported, to our knowledge, in the literature, but a few cases have occurred in Italy.[3,4] For this reason, data concerning

[3]Lodi, F., Institute of Legal Medicine, University of Milan, personal communication, 1988.
[4]Boriello, R., Institute of Legal Medicine, University of Naples, personal communication, 1987.

personally observed cases are useful for gathering information on the levels of unmodified zipeprol in body tissues and fluids.

Our analytical studies have not been able to evaluate the distribution of metabolites because of the unavailability of standards.

The blood concentrations of zipeprol in the two cases we have referred to are about 7 to 14 times higher than the peak plasma level (0.76 μg/mL) observed after a single 175 mg oral dose [5].

The blood concentration of unmodified zipeprol observed in Case No. 1 is about twice that observed in Case No. 2. On the other hand, the amount of zipeprol found in tissues was generally higher in the "pure" case (No. 2) than that found in the mixed intoxication case (No. 1). In the latter, the presence of phendimetrazine and phenmetrazine at toxic levels must be considered a factor contributing to the death and may also explain the different distribution of zipeprol, assuming that sinergy exists between these substances [*13,14*].

Public concern in Italy over the abuse of zipeprol led the Ministry of Health to limit sales of pharmaceutical products containing it by requiring buyers to present a new prescription order each time and by limiting the quantity of the drug that can be sold with each prescription order to one commercial confection (20 or 30 tablets, 100 or 200 mL of syrup, or 10 suppositories).

*Acknowledgments*

The authors wish to thank Fulvia Pozzi for her technical assistance.

## References

[*1*] "Rispat, G., Burgi, H., Cosnier, D., Duchene-Marullaz, P., and Streichenberger, G., "General Pharmacological Properties of a New Non-Opiate Antitussive, Zipeprol (3024 CERM): I. Action on Respiratory Function and Acute Toxicity," *Arzneimittel-Forschung (Drug Research)*, Vol. 26, No. 4, 1976, pp. 523–530.

[*2*] Cosnier, D., Hache, J., Labrid, C., and Rispat, G., "General Pharmacological Properties of a New Non-Opiate Antitussive, Zipeprol (3024 CERM): II. Actions on the Cardiovascular System, Intestinal Transit and Central Nervous System," *Arzneimittel-Forschung (Drug Research)*, Vol. 26, No. 5, 1976, pp. 848–855.

[*3*] Constantin, M. and Pognat, J. F., "Zipeprol Metabolism in Man and in the Animal," *Arzneimittel-Forschung (Drug Research)*, Vol. 28, No. 1, 1978, pp. 64–72.

[*4*] Beckett, A. H. and Achari, R., "A Note on the Metabolism of Respilene in Man," *Journal of Pharmacy and Pharmacology*, Vol. 29, 1977, p. 253.

[*5*] Beckett, A. H. and Achari, R., "Plasma Concentrations and Excretion of Zipeprol in Man under Acidic Urine Conditions," *Journal of Pharmacy and Pharmacology*, Vol. 29, 1977, pp. 589–592.

[*6*] Beckett, A. H. and Achari, R., "Identification of a New Metabolic Product of Zipeprol in Man," *Journal of Pharmacy and Pharmacology*, Vol. 29, 1977, p. 645.

[*7*] *Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Post-Mortem Material*, Clarke, E. G. C., Ed., Pharmaceutical Press, London, 1969.

[*8*] Moroni, C., Cerchiari, E. L., Gasparini, M., and Rota, E., "Overdosage of Zipeprol, a Non-Opiate Antitussive Agent," *The Lancet*, Vol. 1, 1984, p. 45.

[*9*] Perraro, F. and Beorchia, A., "Convulsions and Cerebral Oedema Associated with Zipeprol Abuse," *The Lancet*, Vol. 1, 1984, pp. 45–46.

[*10*] Scuccess, M., "Osservazioni Sullo Zipeprolo come Farmaco d'Abuso," *Bollettino per le Farmacodipendenze e l'Alcoolismo*, Vol. 10, No. 4, 1987, pp. 393–389.

[*11*] Schuch, B., Stephan, U., and Jacobi, G., "Neurotoxic Side-Effects of Piperazines," *The Lancet*, Vol. 1, 1966, p. 1218.

[*12*] Belloni, C. and Rizzoni, G., "Neurotoxic Side-Effects of Piperazines," *The Lancet*, Vol. 2, 1967, p. 369.

[13] Norheim, G., "A Fatal Case of Phenmetrazine Poisoning," *Journal of the Forensic Science Society*, Vol. 13, 1973, pp. 287–289.
[14] Holmgren, P. and Lundquist, O., "Lethal Intoxication with Centrally Stimulating Amines in Sweden 1966–1973," *Zeitschrift für Rechtsmedizin*, Vol. 75, 1975, pp. 265–273.

Address requests for reprints or additional information to
Dr. Orietta Crippa
Istituto di Medicina Legale e delle Assicurazioni
Università di Pavia
Via Forlanini 12
27100 Pavia, Italy