PSILOCYBIN AND PSILOCIN LEVELS IN TWENTY SPECIES FROM SEVEN GENERA OF WILD MUSHROOMS IN THE PACIFIC NORTHWEST, U.S.A.

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Summary

The analysis of twenty species from seven genera of Pacific Northwest mushrooms revealed psilocybin (and in some cases psilocin as well) in seven species from three genera. The species found to contain psilocybin (and psilocin) varied from one collection to another by more than a factor of seven in amount present. Total psilocybin and psilocin levels in species known to be in use for recreational and entheogenic purposes varied from 0.1% by dry weight up to a high of nearly 2% by dry weight.

Introduction

Recreational and entheogenic (Ruck *et al.*, 1979) use of indigenous hallucinogenic mushrooms is very popular in western Washington and Oregon, U.S.A. In the month of October, many pastures are scoured by young people from dawn to dusk in search of *Psilocybe semilanceata* (Fries) Kummer. Landscaped areas, especially where ground bark is used as a mulch, are searched for *Psilocybe stuntzii* Guzmán & Ott, *Psilocybe baeocystis* Singer & Smith, *Psilocybe cyanescens* Wakefield, and other species. Riding stables are visited in search of *Panaeolus subbalteatus* Berkeley & Broome and other species that grow on horse dung and sawdust mixture.

We have frequently been called on to identify mushrooms containing, or thought to contain, psilocybin and psilocin — known hallucinogenic substances. We soon realized that little was known about the amounts of psilocybin and psilocin in the various species. Most chemical studies of Pacific Northwest mushrooms have involved the use of qualitative thin-layer chromatography (TLC) and so have not provided quantitative answers. Tyler (1961) reported the presence of psilocybin in *Psilocybe pelliculosa* (Sm.) Singer & Smith; Benedict *et al.* (1962a) reported on the presence of psilocin but did not find psilocybin in *Psilocybe baeocystis* Singer & Smith; and Benedict *et al.* (1962b) found psilocybin in *Conocybe cyanopus* (Atk.) Kühner, both psilocybin and psilocin in *Psilocybe cyanescens* Wakefield

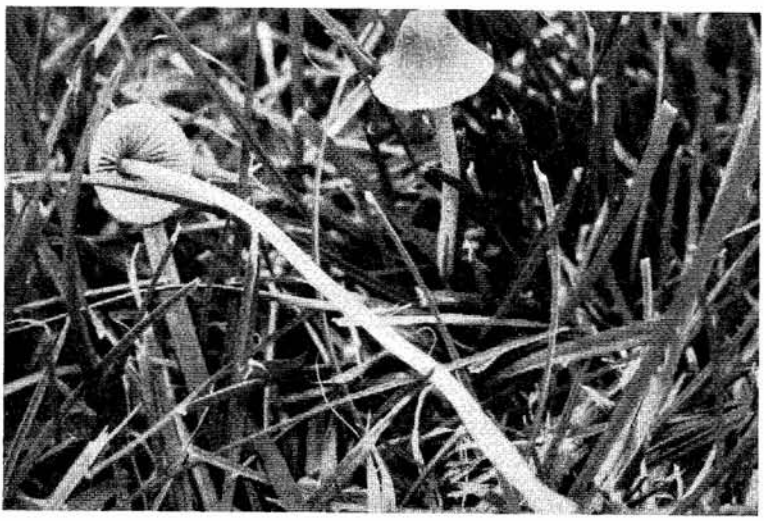

Fig. 1. *Conocybe cyanopus* (Atk.) Kühner. Photo: Bigwood, Washington, U.S.A.

Fig. 2. *Conocybe tenera* (Schaeff. ex. Fr.) Kühner. Photo: Beug, Washington, U.S.A.

Fig. 3. *Conocybe* sp. (near *lactea* (J. Lange) Métrod). Photo: Beug, Washington, U.S.A.

Fig. 4. *Pholiotina filaris* (Fr.) Singer. Photo: Beug, Washington, U.S.A.

Fig. 5. *Panaeotus campanulatus* (Fr.) Quél. Photo: Beug, Washington, U.S.A.

Fig. 6. *Panaeolus acuminatus* (Sec.) Quél. Photo: Beug, Washington, U.S.A.

Fig. 7. *Panaeolus phalenarum* (Fr.) Quél. Photo: Beug, Washington, U.S.A.

Fig. 8. *Psathyrella foenisecii* (Fr.) Smith. Photo: Beug, Washington, U.S.A.

and psilocin with a trace of psilocybin in *Psilocybe baeocystis*. Repke *et al.* (1977) reported on the presence of baeocystin as well as psilocybin and psilocin in six species of *Psilocybe*, two species of *Conocybe*, and in *Panaeolus subbalteatus* (Berk & Br.) Sacc. from the Pacific Northwest. They also reported 4-hydroxytryptamine in *Psilocybe baeocystis* and *Psilocybe cyanescens*.

Using high-performance liquid chromatography (HPLC) we have reported quantitatively on the levels of psilocybin and psilocin in a new species and new variety of *Psilocybe* (Stamets *et al.*, 1980). We have also made a detailed evaluation of HPLC and TLC solvent systems using samples of *Psilocybe baeocystis* Singer & Smith (Beug and Bigwood, 1981). In this paper, we report on the quantitative analysis by HPLC of Pacific Northwest mushrooms believed to contain psilocybin and psilocin. We confirmed the presence of psilocybin and psilocin by TLC analysis for all of our collections.

Experimental

The HPLC system used was a Waters 200 series chromatograph with a 6000A reciprocating pump, a 440 fixed-wavelength absorbance detector at 254 nm, a U6K septumless injector, and a 30 cm × 3.9 mm I.D. μBondapak C_{18} column (particle size 10 μm) using 75% water–25% methanol containing 0.05 M heptanesulfonic acid adjusted to pH 3.5 with acetic acid (Waters PIC B-7 reagent) at 2 ml/min. The runs were recorded on a Hewlett-Packard 3380A reporting integrator–plotter. We found a linear relationship (±10% repeatability) between concentration and peak area from 0.2 to 3 μg total psilocybin or psilocin. The detection limit was about 0.01 μg of psilocybin or psilocin.

Mushroom samples were freeze-dried, sealed in plastic, and stored either at −5 °C or at −60 °C. After grinding, 250-mg portions were stirred at room temperature with roughly 7 ml of methanol for 12 hours, filtered through a 0.5 μm Teflon filter, rinsed with additional methanol, and diluted to exactly 10 ml. An injection volume of 10 μl produced satisfactory peak size at 0.1 a.u.f.s. The instrument was calibrated with psilocybin and psilocin standards obtained from the National Institute on Drug Abuse.

TLC was carried out on oven dried 5 × 20 cm silica gel plates coated with 0.25 mm silica gel $60F_{254}$ (Merck, Darmstadt, G.F.R.). The plates were developed with butanol–acetic acid–water (12:3:5) for a distance of 15 cm at room temperature in the dark, dried and then placed under a short-wave ultraviolet (UV) lamp (Mineralight:UV Products, Inc.). Any spots visible to the naked eye or under short-wave UV were circled. Fresh Ehrlich's or Van Urk's reagent (10% *p*-dimethylaminobenzaldehyde in conc. HCl) was sprayed in an acetone solution (1 part Ehrlich's:4 parts acetone) and the plate was allowed to develop overnight by wrapping it in the paper towel upon which it had been sprayed. Alternatively, we used a dip of 20% pure water-free *p*-toluenesulfonic acid in methanol. Plates were then photographed and stored for future reference.

Fig. 9. *Panaeolus semiovatus* (Fr.) Lundell & Nanfeldt. Photo: Bigwood, Washington, U.S.A.
Fig. 10. *Panaeolus subbalteatus* (Berk. et Br.) Sacc. Photo: Beug, Washington, U.S.A.

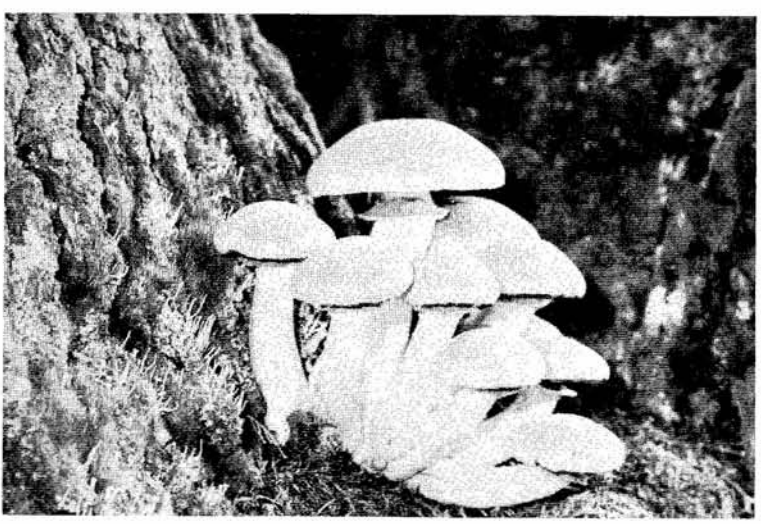

Fig. 11. *Gymnopilus ventricosus* (Earle) Hesler. Photo: Beug, Washington, U.S.A.

Fig. 12. *Stropharia aeruginosa* (Fr.) Quél. Photo: Beug, Washington, U.S.A.

Fig. 13. *Psilocybe semilanceata* (Fr.) Quél. Photo: Beug, Washington, U.S.A.

Fig. 14. *Psilocybe cyanescens* Wakefield. Photo: Beug, Washington, U.S.A.

Fig. 15. *Psilocybe stuntzii* Guzmán & Ott. Photo: Bigwood, Washington, U.S.A.

Fig. 16. *Psilocybe baeocystis* Singer & Smith. Photo: Bigwood, Washington, U.S.A.

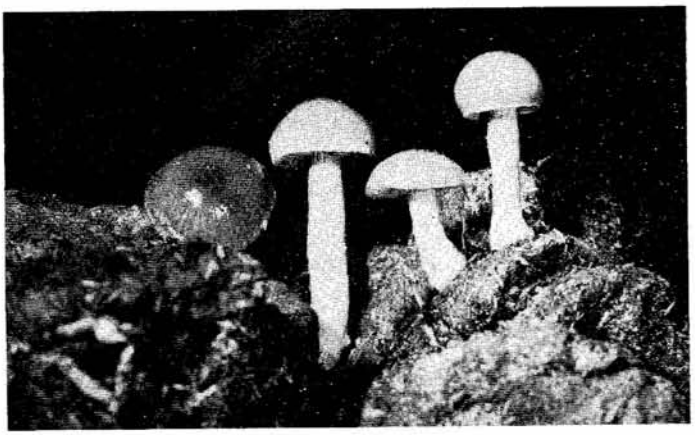

Fig. 17. *Psilocybe coprophila* (Bull. ex Fr.) Kummer. Photo: Beug, Idaho, U.S.A.

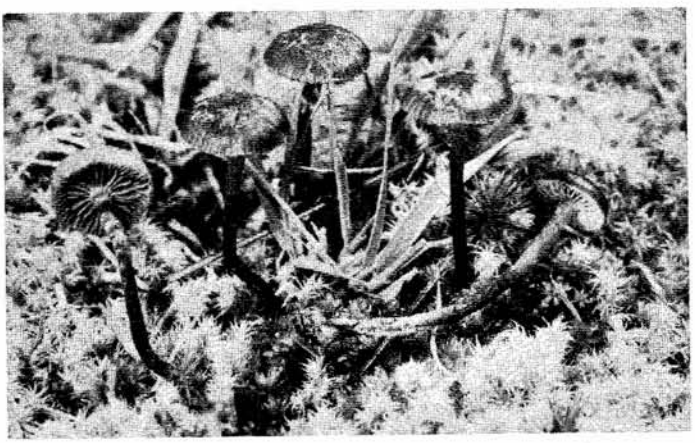

Fig. 18. *Psilocybe montana* (Pers. ex Fr.) Kummer. Photo: Beug, Washington, U.S.A.

Fig. 19. *Psilocybe inquilina* (Fr. ex Fr.) Bres. Photo: Beug, Washington, U.S.A.

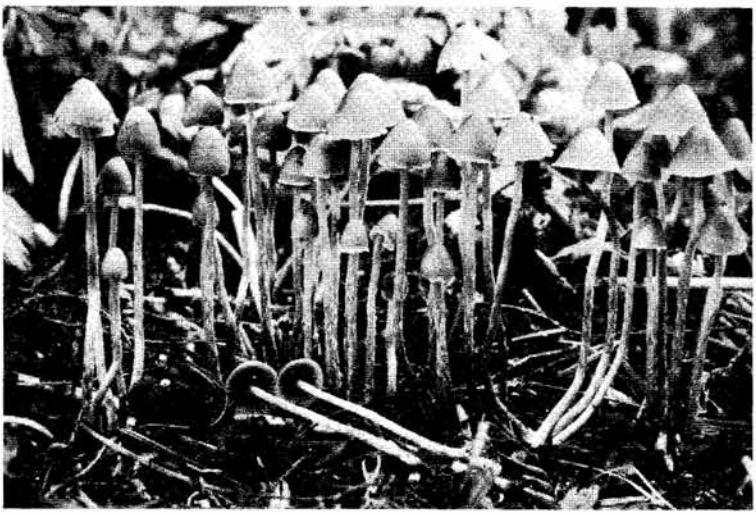

Fig. 20. *Psilocybe pelliculosa* (Sm.) Singer & Smith. Photo: Bigwood, Washington, U.S.A.

Results

We analyzed twenty species from seven genera of Pacific Northwest mushrooms (Figs. 1 - 20) and found psilocybin (and in some cases psilocin as well) in seven species from three genera (Table 1). In many cases, we were able to collect and analyze several samples of the same species and generally found large variations in psilocybin (and psilocin) from one sample to another. The observation of highly variable psilocybin and psilocin levels was consistent with our findings with *Psilocybe cubensis* (Earle) Singer grown under controlled laboratory conditions (see Bigwood and Beug, 1982). In all samples, the presence or absence of psilocybin and psilocin was confirmed by TLC. Tests with a spiking mix revealed greater than 90% recovery of psilocybin and psilocin in a 12-hour extraction at room temperature. Freeze-dried samples showed no detectable loss of psilocybin or psilocin when stored at −5 °C or at −60 °C, but some freeze-dried samples lost both psilocybin and psilocin over periods of one to two years when stored at room temperature. Methanolic extracts were stable for over a year at −5 °C, but within six months lost all psilocin and some psilocybin when stored at room temperature.

The HPLC chromatograms obtained with 75% water −25% methanol with 0.05 M PIC B-7 reagent and a 2 ml/min flow rate on a 30-cm C_{18} column showed psilocybin as a sharp well-resolved peak at 1.97 ± 0.03 minutes and psilocin as a somewhat broadened, but well-resolved peak at 4.8 ± 0.1 minutes. The TLC chromatograms, after development in butanol–acetic acid–water (12:3:5) and visualization with Ehrlich's reagent, showed psilocybin as a well-resolved brownish purple spot at R_f 0.16 and psilocin as a well-resolved darker purple spot at R_f 0.36.

TABLE 1

Psilocybin and psilocin levels in Pacific Northwest mushrooms as quantified by reversed phase HPLC

Species[1] (Figs. 1 - 20)	Date collected[2]	Psilocybin (mg/g dry weight)	Psilocin (mg/g dry weight)
Conocybe cyanopus (Atk.) Kühner	July 12, 1979	9.3	0
Conocybe tenera (Schaeff. ex Fr.) Kühner	Aug. 22, 1978	0	0
Conocybe sp. (near *lactea* (J. Lange) Métrod)	Aug. 13, 1978	0	0
Pholiotina filaris (Fr.) Singer	Oct. 1979	0	0
Panaeolus campanulatus (Fr.) Quél.	Aug. 13, 1978	0	0
	Sept. 4, 1978	0	0
	Mar. 18, 1980	0	0
Panaeolus acuminatus (Sec.) Quél.	Sept. 4, 1978	0	0
	Mar. 18, 1980	0	0
Panaeolus phalaenarum (Fr.) Quél.	July 23, 1978	0	0
	Cultivated	0	0
Psathyrella foenisecii (Fr.) Smith	June 18, 1978	0	0
	Oct. 4, 1978	0	0
Panaeolus semiovatus (Fr.) Lundell & Nanfeldt	June 28, 1978	0	0
	July 12, 1979 (3 collections)	0	0
Panaeolus subbalteatus (Berk. & Br.) Sacc.	July 3, 1978	3.5	0
	Sept. 5, 1978	6.5	0
	Sept. 3, 1979	1.6	0
Gymnopilus ventricosus (Earle) Hesler (generally identified as *G. spectabilis*)	Oct. 15, 1979	0	0
Stropharia aeruginosa (Fr.) Quél.	Oct. 29, 1979	0	0
Psilocybe semilanceata (Fr.) Quél.	Oct. 1979	6.9	0
	Sept. 4, 1978	10.8	0
	Oct. 22, 1979	12.0	0
	Nov. 5, 1979	6.9	0
	Nov. 27, 1979a	8.4	0
	Nov. 27, 1979b	12.8	0
	Nov. 27, 1979c	9.2	0
	Nov. 27, 1979d	6.6	0
	Nov. 27, 1979e	10.9	0
	Nov. 27, 1979f	8.5	0
Caps only	Nov. 27, 1979g	11.1	0
Stems only	Nov. 27, 1979g	6.2	0
Psilocybe cyanescens Wakefield	Oct. 1978a	4.9	1.7
	Oct. 28, 1979	1.5	9.6
	Nov. 2, 1979	8.2	1.3
	Nov. 6, 1979	11.5	7.6

(continued overleaf)

TABLE 1 *(continued)*

Species[1] (Figs. 1 - 20)	Date collected[2]	Psilocybin (mg/g dry weight)	Psilocin (mg/g dry weight)
Psilocybe cyanescens	Nov. 7, 1979a	8.3	2.0
Wakefield	Nov. 7, 1979b	16.8	2.8
	Nov. 18, 1979a	14.3	2.8
	Nov. 18, 1979b	15.5	2.4
	Nov. 18, 1979c	13.6	2.3
	Nov. 18, 1979d	11.0	2.0
	Nov. 18, 1979e	10.1	1.4
	Nov. 18, 1979f	8.7	1.8
	Nov. 18, 1979g	9.7	2.8
	Nov. 19, 1979	8.4	0.6
Psilocybe stuntzii	Sept. 24, 1978	0	0
Guzmán & Ott	Oct. 27, 1979	3.6	0.6
	Oct. 31, 1979	0.4	0.12
	Nov. 13, 1979	3.6	0.06
Psilocybe baeocystis	Sept. 1979a	2.04	1.43
Singer & Smith	Sept. 1979b	1.96	1.32
	Sept. 1979c	1.92	0.48
	Sept. 1979d	2.04	3.07
	Sept. 5, 1979	8.5	5.9
	Oct. 1979a	2.8	0.8
	Oct. 1979b	1.5	0
Psilocybe coprophila (Bull. ex Fr.) Kummer	June 22, 1979	0	0
Psilocybe montana (Pers. ex Fr.) Kummer	Mar. 18, 1980	0	0
Psilocybe inquilina (Fr. ex Fr.) Bres.	Mar. 18, 1980	0	0
Psilocybe pelliculosa	Oct. 30, 1979	7.1	0
(Sm.) Singer & Smith	Oct. 8, 1979	4.1	0
	Nov. 8, 1979	1.2	0

[1]The species were identified by the senior author who has, along with his students, consulted with Dr. Gaston Guzmán on the identification of *Psilocybe*, with Dr. Roy Watling on the identification of *Panaeolus* and *Conocybe*, and with Dr. Daniel Stuntz and Dr. Joe Ammirati on general taxonomic questions. Paul Stamets checked the microscopic features of many of the species.

[2]Voucher specimens were maintained of all species and the collection date was assigned as the collection number. Multiple collections on a single date were indicated by lowercase alphabetical notation. Voucher specimens were prepared for deposit in the University of Washington Herbarium (WTU).

Our analysis included all of the popular hallucinogenic mushrooms species of the Pacific Northwest as well as several species that are frequently collected because they resemble hallucinogenic species or are rumored to be hallucinogenic. *Gymnopilus ventricosus* (Earle) Hesler, a western species which is often mistaken for *G. spectabilis* (Fr.) Smith, a species often found to contain psilocybin, was found not to contain psilocybin, confirming previous reports.

Conocybe cyanopus (Atk.) Kühner, a species with greenish or bluish tints near the base of the stem, was found to contain a significant quantity of psilocybin (9.3 mg/g dry weight). Other non-bluing *Conocybe* species were all found to be lacking both psilocybin and psilocin.

Panaeolus subbalteatus, a non-bluing *Panaeolus* species, was found to contain 1.6 - 6.5 mg/g dry weight psilocybin but no psilocin. This is a popular recreational species, but with the quantity of psilocybin varying by over a factor of four, we wonder what kind of surprises are in store for someone who first samples a relatively weak batch and then increases the dose for effect but is sampling a relatively potent batch? None of the other *Panaeolus* species that we sampled were found to contain either psilocybin or psilocin, though we know that some [especially *Panaeolus campanulatus* (Fr.) Quél.] are sampled from time to time. *Psathyrella foenisecii* (Fr.) Smith, commonly known as *Panaeolus foenisecii* the "haymakers' *Panaeolus*", was also found not to contain psilocybin or psilocin, in agreement with earlier observations, and in contrast to the reports of psilocybin in eastern collections.

Stropharia aeruginosa (Fr.) Quél. is commonly collected by hallucinogen seekers because of its overall bluish-green coloration, but it was found to contain neither psilocybin nor psilocin.

Among the *Psilocybe* species, *Psilocybe semilanceata* (Fr.) Quél., the "liberty cap", a species that blues inconsistently, is most often sought. It is a consistent producer of psilocybin with levels ranging from 6.5 to 12.8 mg/g, typically in excess of 10 mg/g, making it one of the most potent species as well as one of the most constant in amount. It was not found to contain psilocin. *Psilocybe cyanescens* Wakefield, a markedly bluing species, is another popular mushroom (it inhabits landscaped yards containing ground bark rather than meadows and pastures) and is usually even more potent than *Psilocybe semilanceata*. Psilocybin levels were found to range from 4.9 mg/g up to 16.8 mg/g dry weight with total psilocin plus psilocybin levels reaching 19.6 mg/g dry weight — nearly 2% of the dry weight! *Psilocybe stuntzii* Guzmán & Ott, a bluing species that favors yards and playfields and seems to be second in overall popularity because of its abundance, was found to be comparatively weak, sometimes containing neither psilocybin nor psilocin. The maximum we found was 3.6 mg/g psilocybin and 0.6 mg/g psilocin. The real hazard with this species is that users eat a large quantity (often over 50 and frequently over 100 specimens at a time) and could get a very strong reaction if they happened upon a potent collection. There is also concern that they might mistakenly pick the highly similar *Galerina autumnalis* (Pk.) Smith & Singer, a species suspected to contain deadly amanitins, and potentially fatal at these large doses. *Psilocybe baeocystis* Singer & Smith is another popular strongly bluing species that frequents beds of ground bark and is common on school campuses. It generally contains relatively low levels of both psilocybin (up to 2.8 mg/g) and of psilocin (up to 1.4 mg/g), but one collection was found containing 8.5 mg/g psilocybin and 5.9 mg/g psilocin — rivaling the strongest *Psilocybe* species. *Psilocybe pelliculosa*, an infrequently bluing species and the only hallucinogenic *Psilocybe* species

that we find native in the woods, was also quite variable in psilocybin, with levels ranging from 1.2 mg/g to 7.1 mg/g dry weight. It was not found to contain psilocin. *Psilocybe inquilina* (Fr. ex Fr.) Bres., a non-bluing native species that grows on grass stems, was found to contain neither psilocybin nor psilocin. Both compounds were also absent in *Psilocybe montana* (Pers. ex Fr.) Kummer, a non-bluing species found in moss in the spring, and in *Psilocybe coprophila* (Bull. ex Fr.) Kummer, a non-bluing species found on dung in the spring through fall.

Conclusions

The amount of psilocybin and psilocin in collections of Pacific Northwest mushrooms picked for their hallucinogenic effect ranges from just over 0.1% by dry weight to nearly 2% by dry weight. Psilocybin is most often encountered, but psilocin was usually present as well (though psilocin was never detected in the absence of psilocybin).

On the average, *Psilocybe cyanescens* was the most potent species (13 ± 4 mg/g psilocybin plus psilocin) with *Psilocybe semilanceata* (10 ± 2 mg/g average psilocybin) second and *Psilocybe stuntzii* (2 ± 2 mg/g average psilocybin plus psilocin) the weakest of the commonly used species. The levels of psilocybin and psilocin in any one species of hallucinogenic mushroom from the Pacific Northwest were found to be highly variable, varying by a factor of two to more than a factor of six.

Recreational users of hallucinogenic mushrooms sometimes regard the intensity of bluing as a guide to psilocybin and psilocin levels, but we could find no correlation between degree of bluing and either psilocybin or psilocin levels. The variability of psilocybin and psilocin within each species as well as the difference in average level between species leads us to conclude that recreational users of these mushrooms are ingesting unpredictably varying amounts of psilocybin and psilocin. The variability of the bluing reaction further leads us to conclude that this reaction is not a guide to psilocybin or psilocin levels. For several species, we also separately analyzed the cap and various parts of the stem, but generally found that the psilocybin and psilocin were fairly evenly distributed throughout the mushrooms, though the caps sometimes were more potent.

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Note added in proof

On December 16, 1981, three youths picked a *Galerina* species on Whidbey Island, Washington, U.S.A., mistaking it for a *Psilocybe* species. They became ill but did not report their illness for two days for fear of prosecution for use of psilocybin. After subsequent medical treatment, two of the youths recovered but the girl, aged 16, died on December 24, 1981.