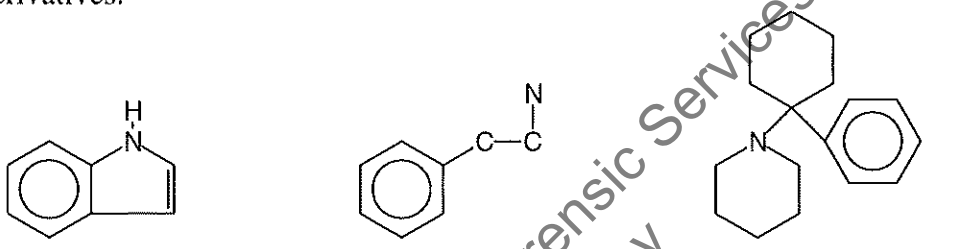
Idaho State Police

Forensic Laboratory Training Manual

Hallucinogens

1.0.0 Backround

Hallucinogens fall in to three general categories - 1) compounds containing the indole skeleton, 2) compounds containing the phenethylamine skeleton and 3) phencyclidine derivatives.



Indole Phenethylamine Phencyclidine (PCP)

Figure 1

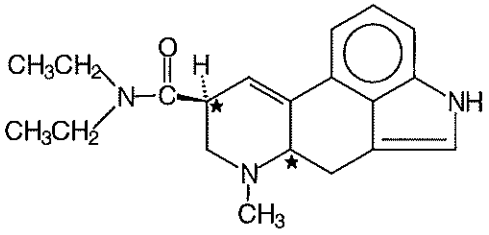
There are several groups within the "indole" category, including ergot alkaloids, and tryptamines.

2.0.0 LSD and other ergot alkaloids

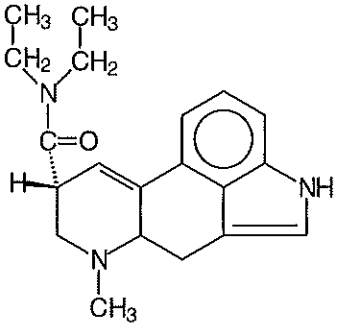
d-Lysergic Acid Diethyl amide Tartrate (LSD) was first synthesized in 1938 by Arthur Stoll and his collaborator, Albert Hofmann, both of the Sandoz Laboratories, Basel, Switzerland. It was not until 1943 that Hofmann, as a result of an accidental ingestion of some of the derivatized material, recognized the potent effects of LSD. Stabilized in the form of tartrate salt, as little as 0.025 mg of LSD was found to cause hallucinations – distortion of perception.

The starting material for the synthesis of LSD is d-lysergic acid, a compound obtained by alkaline hydrolysis of alkaloids produced by a fungus on certain species of rye and other grains and on morning glory and Hawaiian wood rose seeds. From these materials, a mixture of compounds, called ergot, can be extracted. Lysergic acid and other alkaloids isolated from ergot are known as ergot alkaloids. The chemical structures of LSD and lysergic acid contain the indole nucleus, which is also found in the tryptamines.

LSD has two asymmetric carbon atoms in its molecular structure (which are denoted by stars in the structure of LSD in Figure 2). Because of this, there are four possible stereoisomers; d- and l-LSD and d- and l-iso-LSD. Of these four, only d-LSD has a high degree of physiological activity.



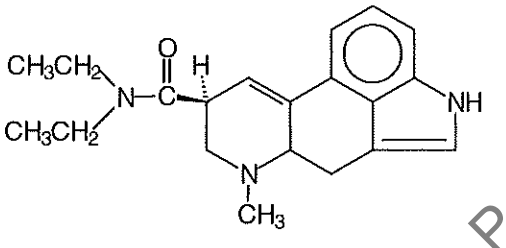
LSD



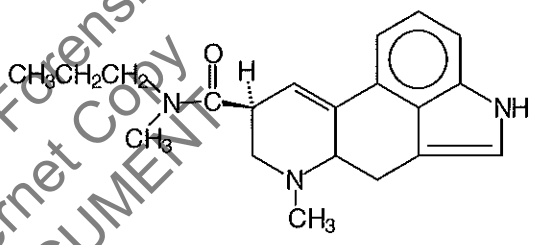
iso-LSD

Figure 2

A compound known as LAMPA (lysergic acid N-methyl-N-propylamide) is structurally very similar to LSD. LAMPA has a mass spectrum and chromatographic properties that are very similar to LSD. The structure of LAMPA is given in Figure 3.



LSD



LAMPA

Figure 3

LAMPA also has two asymmetric carbons so four stereoisomers are possible d- and l-LAMPA and d- and l-iso-LAMPA.

In the illegal market, LSD is usually found deposited on thick paper known as blotter paper which is usually divided by perforations into numerous squares about ¼" per side. LSD is occasionally found intermixed or deposited on strips of gelatin (and cut into squares known as "windowpanes"). In addition, it has been found in the form of very small tablets known as "microdots" or "barrels", and occasionally mixed into various food preparations.

The small quantity of LSD present in dosage preparations presents some analytical difficulties. In tablet preparations, the drug is extensively diluted with sugars, starches, binders, lubricants, gelatin, and other excipients. It is not uncommon to find the active ingredient representing only 0.1% of the total dosage weight.

2.1.0 Screening tests

Tests that can be used to check for the presence of LSD include spot tests and UV fluorescence. A spot test widely used for the detection of compounds with an indole nucleus employs the use of Van Urks reagent (refer to section on spot tests for preparation of Van Urks reagent). In the presence of an ergot alkaloid, this light

yellow reagent produces a violet to purple color. Note that LSD tablet preparations frequently contain dyes that can obscure this color or produce a false color reaction.

LSD will fluoresce under long-wave UV light. A quick screening test for LSD can be accomplished by spotting a methanol extract of the suspected sample on a TLC plate, placing it under long-wave UV light and looking for a fluorescent "spot". Occasionally, dyes from the blotter paper or tablet preparations will also fluoresce. LSD is so highly fluorescent that in many instances, the fluorescence from dyes is insignificant by contrast. Development of the TLC plate will separate the LSD from these compounds.

2.2.0 Extractions

In most instances LSD can be dry extracted from the sample by simply soaking the evidence in a small amount of methanol for about 10 minutes. In certain cases, methanol-soluble excipients may interfere with the analysis procedure. This will most likely be a problem with TLC. To circumvent this problem, the sample may be subjected to a double extraction procedure in which a sample portion is dissolved in 2% citric or tartaric acid solution and extracted with chloroform. These extracts containing the impurities are discarded. The acidic solution is rendered alkaline with solid sodium bicarbonate and the liberated LSD base is extracted with chloroform. After evaporating the extract, the resulting reside is redissolved with a few drops of chloroform and subjected to analysis.

2.3.0 GC/MS

For a number of years, the low volatility of LSD and its tendency to decompose at high temperatures precluded the use GLC in the analysis of LSD. With the advent of inert GC injectors and capillary columns coupled with high sensitivity mass spectrometers, analysis of LSD via GC/MS has become common. Low polarity columns such as methyl-silicon work the best, but high GC temperatures are still necessary. Great care must be taken when analyzing LSD via GC/MS as LAMPA gives a mass spectrum that is very similar to LSD and, depending on GC conditions, a retention time that is near that of LSD.

Introduction into the MS can also be accomplished via a solid probe. Cleanup of the sample prior to MS analysis is necessary, however techniques such as MS/MS can greatly reduce the amount of cleanup.

Hybrid techniques such as chemical ionization with ammonia reagent gas coupled with MS/MS have been developed to lower the levels of detectability of LSD. These techniques are especially useful for toxicology analyses.

2.4.0 TLC

A great deal of difficulty has been reported in separating LSD from LAMPA by TLC. Developing solvents that contain acetone - such as 100% acetone or a 1 to 1 ratio of acetone and chloroform can separate the two compounds. Visualization of the TLC

plates can be accomplished by fluorescence under long-wave UV or by spraying the plates with Van Urks reagent.

2.5.0 IR

FTIR can and has been used for the identification of LSD: however, a rigorous cleanup procedures such as preparative TLC is required. Frequently, special techniques such as the use of micropellets are necessary because of the small amount of LSD present in dosage forms.

2.6.0 HPLC

HPLC gives the best chromatography for LSD. LSD is highly fluorescent making the UV fluorescent detectors found on many HPLC's well suited for detecting LSD. These two factors make HPLC a very sensitive method for detecting LSD, and the method of choice for quantitating LSD. Because UV fluorescent detectors cannot distinguish between LSD, iso-LSD and LAMPA, chromatographic conditions must be carefully selected.

Adequate quantities of LSD can be isolated for IR or solid probe MS by collecting the eluantes from HPLC.

LSD is highly fluorescent in acid solution. Although a fluorescence spectrum is not specific for LSD, if the excitation and emission spectra of a sample solution corresponds to those of standard LSD, one may strongly suspect LSD to be present.

3.0.0 Tryptamines

The tryptamines are structurally similar to Serotonin and Melatonin that are naturally present in the human body. The most commonly encountered compounds within the tryptamine category include Psilocyn and Psilocybin which are found in mushrooms of the *Psilocybe* genus, and Bufotenine which is found in the skin of *Bufo Marinus* the Australian cane toad, and in the parotid glands on the back of *Bufo alvarius* toads, as well as the seeds of the genus *Anadenanthera* (*piptadenia*) such as *piptadenia peigrina*, and the bark of the genus *Banisteriopsis* (*Malpighiacae*).

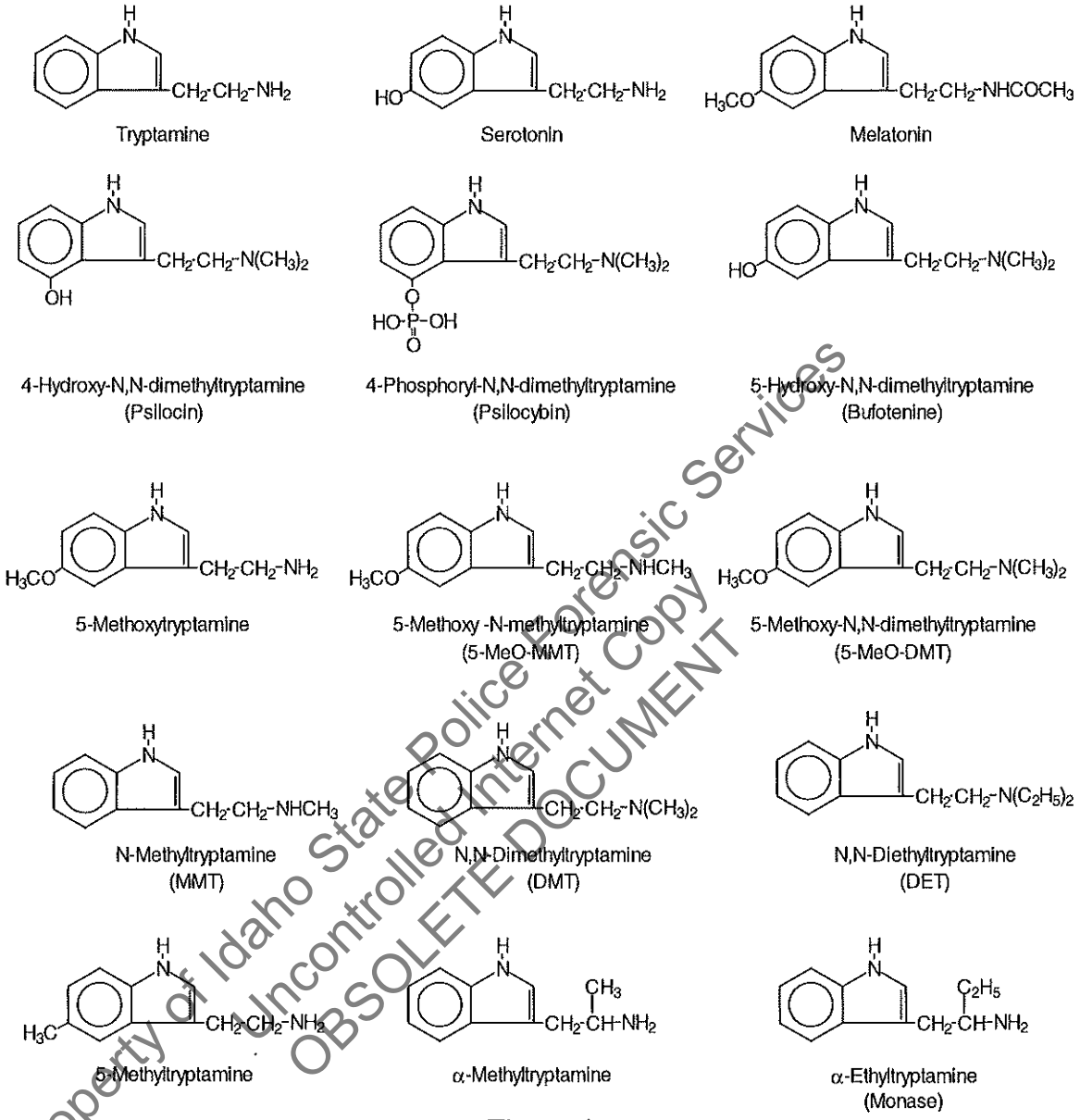


Figure 4

Figure 4 gives the structures of the more common tryptamines.

3.1.0 Spot tests

A spot test widely used for the detection of compounds with an indole nucleus employs the use of Van Urks reagent. In the presence of a tryptamine, this light yellow reagent produces a violet to purple color.

3.2.0 GC/MS

GC/MS is generally suitable for identifying the tryptamines, although some care must be taken as some of the tryptamines (such as Psilocyn and bufotenine) produce similar mass spectra.

Reference 8 gives more information as well as mass spectral data for some common tryptamines.

4.0.0 Psilocyn and Psilocybin

Psilocyn and Psilocybin are found in mushrooms of the Psilocybe genus. The word Psilocybe comes from the Greek work "psilos" which means bald head. Common species include *Psilocybe cubensis*, *Psilocybe cyanescens, Psilocybe mexicana* and *Psilocybe tampanensis*. The dried mushrooms contain 0.2-0.4% psilocybin and trace amounts of Psilocyn. Psilocybin is a stable water-soluble compound. Psilocyn is water insoluble and easily oxidized. Psilocyn is 1.5 times more potent then psilocybin. A typical dose is 4 to 8 mg. corresponding to about 2 grams of dried mushrooms.

4.1.0 Spot tests

Van Urks reagent can be added directly to dry mushroom material. Due to the low Psilocyn and psilocybin content present in mushrooms, several minutes may elapse before a faint violet to purple color forms.

4.2.0 Extraction

One half to one gram of mushrooms should be crushed or ground and soaked in a minimal amount of methanol for at least five minutes (NOTE: Attempts to concentrate extracts via heating usually result of decomposition of the Psilocyn and psilocybin and should be avoided if possible). The addition of a 1 to three drops of ammonium hydroxide will assist in the extraction as will gentle heating. After soaking for a suitable period of time the methanol is decanted and filtered through a Pasteur pipette with a cotton plug. The solution is ready for analysis.

4.3.0 GC/MS

The heat of the GC injector cleaves the phosphoryl ester of psilocybin turning it into Psilocyn. Hence, Psilocyn and psilocybin give identical results when analyzed via GC/MS.

4.4.0 TLC

TLC using ammonical methanol, as a developing solvent will distinguish Psilocyn from psilocybin. Van Urks reagent makes a suitable visualizing agent.

5.0.0 Substituted Amphetamines and Phenethylamines

Drugs of the ring substituted amphetamine and phenethylamine class have a high potential for abuse and occur frequently on the illicit market. While some of these compounds such as Mescaline are naturally occurring, most are synthetic and constitute a major portion of the "Designer Drugs" on the market. These compounds are often synthesized in an attempt to circumvent controlled substance statutes. Recently, analog laws have been introduced in an attempt stifle this activity. In general, to be declared an analog, the substance must have a chemical structure which is substantially similar to a controlled substance, and: A) ...which has a ... effect on the central nervous system substantially similar to ... a controlled substance, or B)

…which the individual represents to have a … effect on the central nervous system substantially similar to … a controlled substance.

As shown in Figure 5, there are many possible sites on the phenethylamine structure where substitution can take place, and often more than one of these sites have substitutions on them. Methoxy-substituted amphetamines possess both stimulant and hallucinogenic properties.

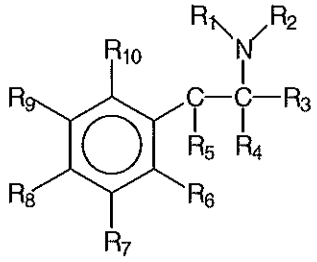
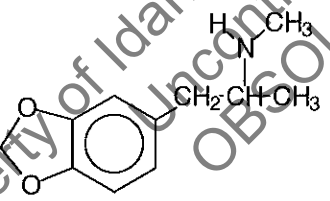
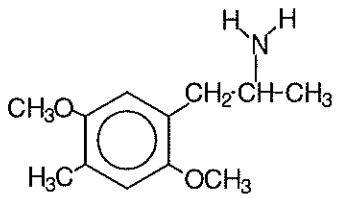


Figure 5

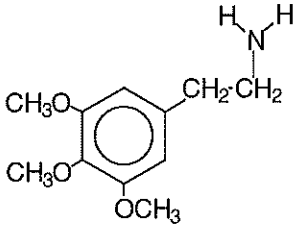
Substituted amphetamines having one, two, or three methoxy groups have all been encountered. Other variations include the addition of a bromine on the ring, e.g., 4-bromo-2,5-dimethoxyamphetamine (Bromo-STP or DOB), the inclusion of methyl group on the ring, e.g., 4-methyl-2,5-dimethoxyamphetamine (STP or DOM), the addition of a methyl group on the nitrogen, e.g., 2-methoxy-N-methylamphetamine (methoxyphenamine), or the shortening of the alkyl side chain to two carbons instead of three, e.g., 3,4,5-trimethoxyphenethylamine (mescaline). Closely related are compounds having oxygen attached to adjacent aromatic carbons and having the oxygen joined by a methylene group, e.g., 3,4-methylenedioxyamphetamine (MDA). Figure 6 shows the structure of some of the more common compounds in this class.



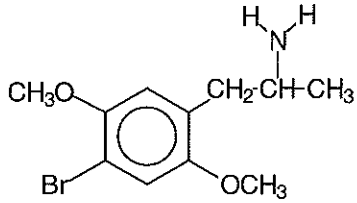
3,4-Methylenedioxymethamphetamine
(MDMA)



2,5-Dimethoxy-4-methylamphetamine
(STP, DOM)



3,4,5-Trimethoxyphenethylamine
(Mescaline)



4-Bromo-2,5-dimethoxyphenethylamine
(Nexus)

Figure 6

5.1.0 Extraction

Since all the substituted amphetamines are strong amines, they can be isolated from neutral and acidic impurities by the usual acid-base extraction techniques. The free bases occur as volatile, oily liquids, making isolation as a salt or a less volatile derivative a necessity.

Presumptive tests that can be used to determine the presence of a methoxy-substituted amphetamine include spot tests (such as the Marquis Test), GLC, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), and UV spectroscopy. Since there is overlap of the responses of the various methoxy-substituted amphetamines, additional instrumental tests may be necessary to determine the identity of the positional isomer present.

5.2.0 GC/MS

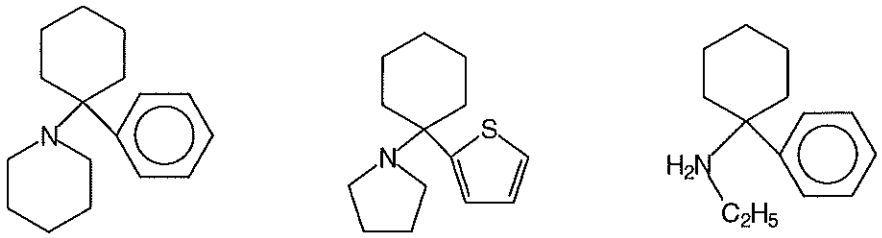
MS has several drawbacks when used to identify methoxy-substituted amphetamines. The spectra obtained are weak, and amphetamines with the same molecular weight exhibit very similar fragmentation patterns. MS alone may not allow identification of the substitution pattern. The molecular ions are of low intensity, and most ring-substituted amphetamines have a base peak of *m/z* 44 while most N-methylamphetamines have a base peak of *m/z* 58.

5.3.0 IR

Infrared Spectrophotometry (IR) permits the unequivocal identification of all the substituted amphetamines. Both the free bases and salts can be used for the identification. Since even the salts tend to be hygroscopic, considerable difficulty is frequently encountered in obtaining a KBr dispersion disc suitable for use. The easily prepared and less hygroscopic phenylisothiocyanate derivatives are suitable for overcoming this obstacle.

6.0.0 PCP and its Analogs

The scheduling of the animal tranquilizer phencyclidine (PCP) as a controlled substance in 1970 brought to the illicit market a number of analogs reported by Maddox, et. al., to give responses in animals similar to those produced by PCP. One such compound in 1-[1-(2-thienyl) cyclohexyl] piperidine (TCP), in which the thienyl group has replaced the phenyl group in PCP. Another analog is PCE, in which N-ethyl has replaced the piperidine ring in PCP. (see Figure 7).



PCP TCP PCE

Figure 7

6.1.0 Extraction

Since PCP and its analogs are basic, they can be isolated from neutral and acid impurities by the usual acid-base extraction techniques. Phencyclidine hydrochloride is very soluble in $CHCl_3$ and can be easily extracted by $CHCl_3$ from an aqueous solution containing chloride ions. The free bases of PCP and its analogs are either liquids or low melting solids. Their HCl salts are stable solids but are hygroscopic to a varying degree.

Screening tests that can be used to detect the presence of PCP or its analogs include UV, TLC, and GLC.

6.2.0 TLC

A wide variety of solvent systems and absorbents have been used for the TLC identification of PCP and its analogs.

6.3.0 GC/MS

Phencyclidine and its analogs are also sufficiently thermally stable to be identified by GLC on a wide variety of columns. Injection of the free base is preferable because of some decomposition upon injection of the HCl salts. Those analogs having the thiophene moiety are less thermally stable than those having the phenyl moiety. The EI mass spectra of phencyclidine and its analogs are very informative. They can be used to confirm a suspected structure, as well as to elucidate the structure of uncharacterized analogs of PCP. The molecular ions vary in intensity but can be assigned, nonetheless. Other prominent mass fragments include M - 83 in the thiophene series and M - 77 in the phenyl series, resulting from loss of the respective unsaturated moieties. All members of the thiophene series give a strong peak at m/z 97, while those of the phenyl series give a strong peak at m/z 91. These are characteristic of the thiophene and benzene derivatives. Phencyclidine and its analogs that contain the cyclohexyl moiety also give a strong M-43, resulting from the loss of a C_3H_7 radical portion of this ring.

6.4.0 IR

The IR spectra of both the free bases and the hydrochloride salts can be used for identification. Both are capable of distinguishing closely related isomers and homologs.

Because of the large number of PCP analogs that can and have been prepared, multiple instrumental tests may be necessary to establish the complete identity of the compound present. These tests include MS and IR.

7.0.0 Exercises

1. Analyze LSD and LAMPA via GC/MS, and TLC. Which techniques differentiated LSD from LAMPA and describe the differences.
2. Obtain mass spectra of LSD, LAMPA. Can any differences be observed in the spectra? What conclusions can be drawn from this exercise for the identification of these compounds?
3. List the mass spectral differences between psilocyn and bufotenine.
4. Read the ISP SOP and list the requirements for reporting Psilocyn and/or psilocybin was detected on a lab report.
5. List the substituted phenethylamines that are controlled under Idaho Statutes.

8.0.0 Questions

1. Why does the analysis of LSD present a significant health hazard to the forensic chemist and what precautions must be taken when analyzing. LSD?
2. Describe a rapid method for the detection of LSD on your hands.
3. An investigator informs you that a suspect has been purchasing large quantities of morning glory seeds. What would you think that the suspect is attempting to do?
4. Is 4-chloro-2,5-dimethoxyamphetamine controlled?
5. A PCP sample is analyzed by GC/MS and found to contain 1-phenylcyclohexene. Can any conclusion be drawn regarding the method of synthesis used to make the PCP?
6. What is the main safety hazard that a chemist faces when synthesizing PCP? At what point in the synthesis does this hazard occur, and what steps should be taken for protection?

9.0.0 Bibliography

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