PINE OIL TOXICITY IN THE HORSE: DRUG DETECTION,
RESIDUES AND PATHOLOGICAL CHANGES

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ABSTRACT

This report concerns the detection and acute toxicity of pine oil (a commercially available disinfectant) after intravenous administration in horses. α Terpineol was identified as a major constituent of pine oil. α Terpineol was recovered from equine tissues by extraction into heptane and detected by gas chromatography, using either flame ionization detection or pentafluoropropionic anhydride derivatization and electron capture detection. After intravenous injection of 0.1 ml/kg, death due to massive pulmonary edema occurred within minutes. In this animal blood and tissue levels of α-terpineol of between 150 and 300 ppm were observed. After smaller doses of pine oil (0.033 ml/kg), horses survived until euthanized up to 48 hours later. Blood levels of α-terpineol became undetectable in one of these animals after 2 hours, and no tissue levels were detected at postmortem. Marked histopathological changes were seen in the lungs of animals which survived the initial injection period. It appears that after intravenous injection of pine oil in horses lesions are largely related to the respiratory tract, and the mechanism of death is acute pulmonary edema.

INTRODUCTION

A suspected case of acute intoxication in a Thoroughbred horse by

intravenous injection of pine oil, a commercially available disinfectant

(Gleason *et al.*, 1969), was brought to us for evaluation. The tentative

diagnosis of pine oil poisoning was based on (a) reported blood and tissue

levels of α-terpineol and (b) postmortem changes in tissues from the horse.

Because little information was available in the literature on this subject

(Horning *et al.*, 1975), we initiated an experimental investigation of pine

oil toxicity in the horse. The results show that doses of pine oil of about

0.1 ml/kg in the horse are rapidly fatal, producing massive pulmonary edema
and death within minutes. Doses of pine oil insufficient to produce death
from acute pulmonary edema are rapidly cleared from the body of the horse
but associated with longer term pathological changes in the lungs.

MATERIALS AND METHODS

Chemical. α Terpineol was obtained from Eastman Kodak, Ltd., Rochester,
New York, and was 99% pure. Pine oil was from the Dimesdt Chemical Company,
Louisville, Kentucky, and was a commercially available disinfectant brand.
Pentafluoropropionic anhydride (PFPA) was from the Pierce Chemical Company,
Rockford, Illinois. All other chemicals were reagent grade. Recovery of
α-terpineol from tissues was by rotoracking in the presence of heptane. α
Terpineol was identified by chromatography in a Perkin-Elmer 900 gas chromato-
graph equipped with a flame ionization detector (FID). Other samples were
derivatized with pentafluoropropionic anhydride (PFPA) as described by Blake
et al. (1973) and chromatographed on a Varian 2740 equipped with an electron
capture detector.

Biological. The horses used in these experiments were yearling Thorough-
bred "wobblers" of about 300 kg scheduled for euthanasia in association
with another research program. Except for signs of locomotor ataxia, these
animals were clinically normal. Pine oil was administered by rapid intra-
venous injection into the jugular vein. Horse No. 1, injected with 0.1 ml/kg
pine oil (about 30 ml), went down at 35 seconds post-dosing and was dead at
4 minutes. This animal was immediately necropsied and samples taken for
histological and toxicological examination. Horse No. 2, dosed with 0.033
ml/kg (about 10 ml) of pine oil, showed transient signs of CNS excitement,

incoordination, and respiratory distress between 1 and 5 minutes after

dosing but thereafter was clinically normal. Blood samples were taken

from this animal at various intervals over the first 24 hours. This animal

was euthanized at 24 hours and tissue samples taken for toxicological and

histopathological analysis. In addition, two other horses were dosed with

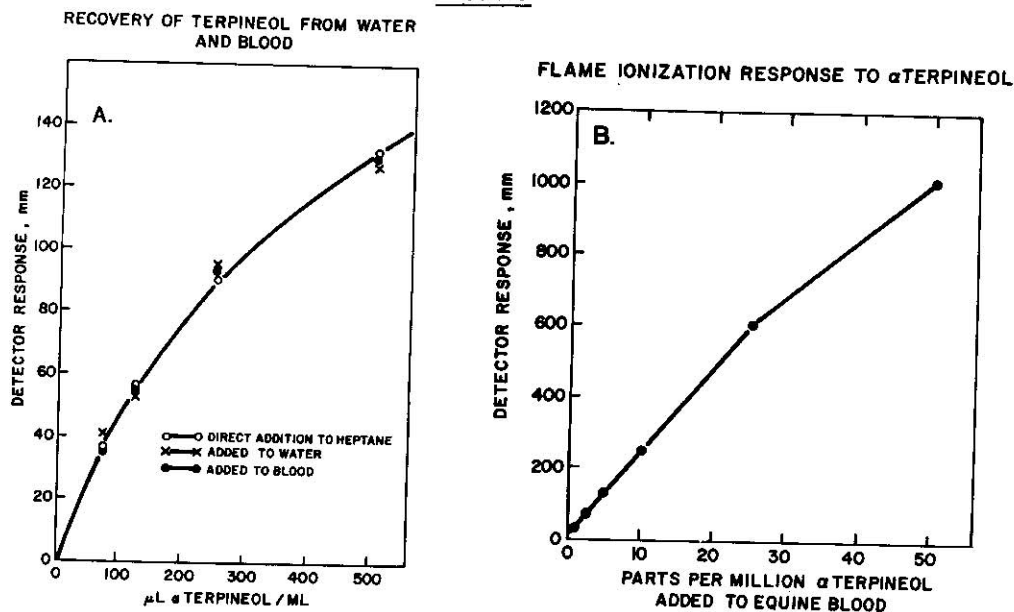0.033 ml/kg of pine oil and necropsied at 24 and 48 hours post-injection.

RESULTS

Fig. 1. Recovery of α-terpineol from water and blood.

Panel A: To 10 ml aliquots of distilled water, freshly drawn equine
blood or 2 ml of heptane the indicated quantities of α-terpineol were
added. The samples were then rotoracked with 2 ml of heptane and
the heptane fractions separated. The open circles (0 - 0) show the
flame ionization response after direct addition of the α-terpineol
to heptane; the crosses (X-X) and the solid circles (●-●) the
responses to α-terpineol recovered from the spiked aqueous and blood
samples. Panel B shows that concentrations of α-terpineol as low
as 1 pmm in equine blood are detectable by this method.

Rotoracking of tissue homogenates with an equivalent volume of heptane allowed quantitative recovery of α-terpineol added directly to tissue samples (Fig. 1, Panel A). Panel B shows that this method is quantitative and sensitive down to about 2 ppm, sufficiently sensitive for toxicological experiments.
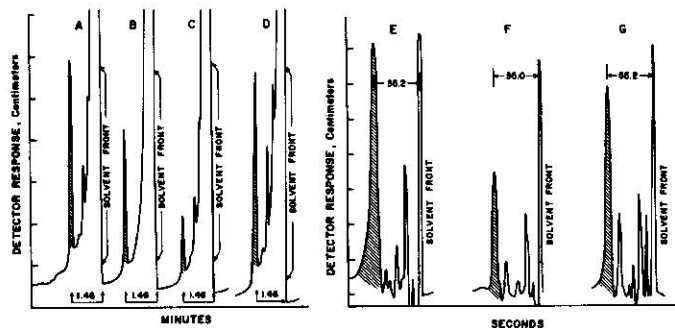

Fig. 2. Chromatograms of pine oil α-terpineol and tissue extracts.

A. α-terpineol in blood
B. α-terpineol in water
C. pine oil
D. renal extract

Perkin-Elmer 900 flame ionization detector, OV101 column, temp. 105°

E. renal extract
F. pine oil
G. α-terpineol

Varian 2740, PFPA derivatives, electron capture detection, OV101, temp. 150°

Figures 2 and 3 identify α-terpineol as the major peak in the commercial pine oil used in these experiments and demonstrate its recovery from an experimentally poisoned horse (Horse No. 1). Chromatograms A, B, and C of Fig. 2

show that α-terpineol and the major peak in pine oil have the same retention times on OV 101 in the Perkin-Elmer 900. Similarly, the major peak recovered from the renal sample also showed a 1.46-minute retention time.
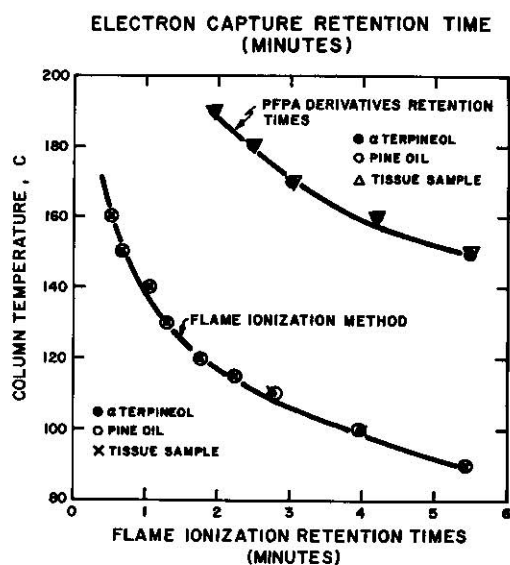

Fig. 3.   Identification of major pine oil peak as α-terpineol.

About 900 μg of α-terpineol or pine oil was added to 10 ml heptane and chromatographed with a heptane extract of the kidney of Horse No. 1 on the Perkin-Elmer 900. The symbols in the lower left hand quarter show retention times over a 70° temperature range for α-terpineol (●), the major pine oil peak and (O) the major peak recovered from the kidney of Horse No. 2 (X). In the upper right hand corner of this figure 100 ppm solutions in heptane of α-terpineol, pine oil and a heptane extract of the kidney of Horse No. 2 were derivatized with PFPA and chromatographed at the indicated temperatures. The symbols (● ,O,Δ) show the retention times of the indicated derivatives over a 40° temperature range.

When these samples were derivatized with PFPA as described by Blake *et al.* (1973) and chromatographed on a Varian 2740 gas chromatograph equipped with an electron capture detector similar retention times were again observed. Figure 3 shows that these peaks eluted with similar retention times over a 70° and 40° temperature range in each system, respectively. These results show that α-terpineol is a major constituent of commercially available pine oil and that this peak is readily recovered from equine tissues shortly after intravenous injection of pine oil in horses. This and other data showed that 150 ppm α-terpineol was found in blood and renal tissue and 300 ppm in lung tissue in Horse No. 1, euthanized with 0.1 ml/kg of pine oil.
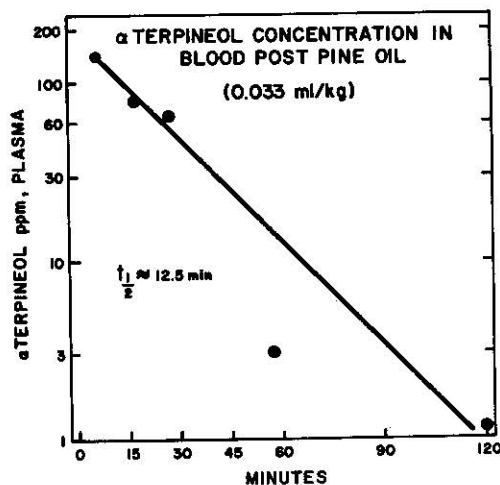

Fig. 4 Bl d levels of α-terpineol after intravenous injection of pine oil.

A Thoroughbred colt, weighting 350 kg, was injected intravenously with pine oil, 0.033 ml/kg. The solid circles (●-●) show blood levels of α-terpineol observed at the indicated times.

Figure 4 shows the levels of α-terpineol observed in the plasma of Horse No. 2 injected with 0.033 ml/kg of α-terpineol. α-Terpineol levels declined with an apparent half-life of about 12.5 minutes. α-Terpineol was no longer

detectable in the plasma of this horse after the 2-hour period and α-terpineol
was not recovered from any of the tissues of this horse after necropsy at 24
hours. Tissues of the other horses necropsied at 24 and 48 hours were not
analyzed for α-terpineol.

After rapid intravenous injection of 0.1 ml/kg of pine oil into the jugu-
lar vein, Horse No. 1 became excited and incoordinated at about 35 seconds and
then went down. The animal showed brief extensor rigidity, tonic clonic con-
vulsions and respiratory distress. At 90 seconds post-injection, the animal
lay as though anesthetized but eyelid and corneal reflexes were still present.
Thereafter, the appearance of the animal improved and it appeared as though
the animal might get up again. However, at about 4 minutes post-injection
the animal experienced respiratory difficulty and died. Postmortem examination
showed very hemorrhagic, congested, and edematous lungs which failed to
collapse. An odor of pine oil was readily detectable. On opening into the
trachea and bronchi these were found to be filled with blood-tinged froth.
Histologic examination revealed severe alveolar congestion and alveolar edema.
The other major organs were histologically normal.

Horse No. 2 was injected with 0.033 mg/kg of pine oil, intravenously, into
the jugular vein. This horse showed signs of respiratory difficulty for 3-
5 minutes and was slightly excited and incoordinated. An odor of pine oil was
detectable on its breath at this time. The horse rapidly returned to normal
and was euthanized 24 hours later.

On postmortem, gross lesions were restricted to the lungs. On opening
into the thoracic cavity the lungs did not collapse. Their ventral portions
were firmer than the dorsal parts and darker in color. There was an intra-
septal edema in the ventral portions of the lungs, principally in the apical
lobes.

Histopathology showed patchy interstitial pneumonia throughout the lungs with some areas more severely affected than others. These areas showed alveolar congestion and edema with fibrin formation. The alveolar macrophages were pleomorphic and proliferating in large patchy areas. Other areas of the lung were less affected. The only histopathological changes observed in other organs was a mild segmental degeneration of some renal epithelial tubular cells.

This lower dose of pine oil was administered to two other horses and similar results observed.

DISCUSSION

α Terpineol was readily recovered after addition to blood and aqueous samples by rotoracking with heptane. It was identifiable in these heptane extracts by flame ionization detection or after derivatization with PFPA to give an electron capturing derivative. By comparing retention times with authentic α-terpineol, the major peak of pine oil and the major peak recovered from animals injected with pine oil were identified as α-terpineol and shown to be recoverable by the methods outlined from animals poisoned with pine oil.

After intravenous injection of 0.1 mg/kg of pine oil into the jugular vein of horses, death occurred within minutes and was apparently due to massive pulmonary edema. In the animals' kidney, lung, and blood levels of between 150 and 300 ppm of α-terpineol were readily identified. Further, the odor of α-terpineol from the lungs of this animal on postmortem and from other tissue samples taken from this animal was marked. Thus, it appears

likely that in cases of acute poisoning of animals by the intravenous in-
jection of pine oil that both the acute postmortem changes, odors, and the
amounts of α-terpineol present would make poisoning in this way relatively
easy to identify.

When a smaller dose of pine oil (0.033 mg/kg) was given, the clinical
signs and pathological changes were quite different. At the lower dose
level transient signs of central nervous system excitation and incoordin-
ation were seen between 1 and 4 minutes post-injection, corresponding with
transiently high blood and CNS levels of pine oil. Again, an odor of pine
oil was identifiable on the breath. However, these signs soon passed and
the animals were subsequently clinically normal. These animals were
euthanized at 24 and 48 hours post-dosing and the tissues of one of these
animals analyzed for α-terpineol.

The data of Fig. 3 show that blood levels of α-terpineol declined
rapidly after its intravenous injection and were no longer detectable at
three hours post-injection. Similarly, no α-terpineol was detected in
tissue samples taken from these horses at postmortem. This is presumably
due to the redistribution and metabolism of α-terpineol and suggests that
α-terpineol is not likely to remain detectable in the tissues of horses
for long periods.

These experiments are in good agreement with those of Hill *et al.*,(1975)
who studied a case of recurrent pine oil poisoning in a child. These investi-
gators were first alerted to the possibility of pine oil toxicity by the odor
of the infant's breath. α-Terpineol poisoning was diagnosed from the presence
of its metabolite, p-menthan-1,2,8-triol in the infant's urine. This same
metabolite was identified in the urine of rats administered pine oil or

α-terpineol and appears to be a major metabolite of α-terpineol. It is likely that to diagnose α-terpineol ingestion in the horse a number of hours after dosing one would have to isolate and identify its metabolites, as α-terpineol itself appears to be rapidly metabolized.

After oral ingestion, pine oil is reported to cause irritation to the mucous membranes and eyes, hemorrhagic gastritis, irritation of the genito-urinary tract, and CNS depression (Gleason et al., 1969). However, by the intravenous route in the horse, the lesions appear to be largely confined to the lungs. A sufficiently large dose (0.1 ml/kg), such as that given to Horse No. 1, will apparently produce death very soon after injection by means of an acute chemical pneumonia. In this case, massive pathology in the lung is seen and the odor of pine oil is marked. However, if the dose of pine oil is reduced the animal escapes death from acute pneumonia but longer term effects are seen in the lung. It appears likely that these lower doses will not directly produce death. Because the pneumonic changes seen at this point are not pathognomic, a diagnosis of sub-acute pine oil poisoning would presumably depend on the identification of metabolites of α-terpineol in the body fluids of the horse since the α-terpineol itself is metabolized in the first three hours.

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