A Species Comparison of the Toxicity of Nabilone, a New Synthetic Cannabinoid

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A Species Comparison of the Toxicity of Nabilone, a New Synthetic Cannabinoid. HANASONO, G. K., SULLIVAN, H. R., GRIES, C. L., JORDAN, W. H., AND EMMERSON, J. L. (1987). Fundam. Appl. Toxicol. 9, 185–197. Acute, subchronic, and chronic studies were conducted in various species to evaluate and compare the toxicity of nabilone, a new synthetic 9-ketocannabinoid that is orally effective for the treatment of nausea and vomiting induced by cancer chemotherapy agents. The oral LD50 in mice and rats for nabilone formulated as a polyvinylpyrrolidone (PVP) codispersion was in excess of 1000 mg/kg. Among nonrodents, rhesus monkeys had a higher tolerance to the CNS depression induced by single oral doses of nabilone-PVP than did dogs. Rats fed dietary mixtures of nabilone-PVP which provided approximate daily nabilone doses of 1 to 93 mg/kg tolerated treatment for 3 months with no deaths. Treatment-related changes (at doses ≥5 mg/kg) were limited to reduced body temperature, slight-to-moderate decreases in weight gain, and behavioral changes (e.g., hyperactivity, hyperirritability to touch, and hypoactivity). All dogs treated for 3 months with daily oral doses of up to 1.0 mg/kg survived; treatment-related effects were limited to transient episodes of ataxia and anorexia. Nabilone treatment of rats and dogs for 3 months produced no evidence of systemic toxicity in the clinical chemistry, hematology, or pathology parameters examined. Chronic treatment of dogs with daily oral doses of nabilone-PVP equal to 0.5, 1.0, or 2.0 mg of nabilone/kg produced cumulative toxicity; by the end of 7 months, 2, 6, and 7 dogs in the respective dose groups had died. In a number of instances, death was preceded by one or more convulsive episodes. In contrast to the dog, the toxic potential of nabilone was minimal in rhesus monkeys treated with nabilone-PVP for 1 year at daily oral nabilone doses of up to 2.0 mg/kg. The enzymatic reduction of the 9-keto group of nabilone to form carbinol metabolites was a major metabolic pathway for nabilone in dogs but not in rhesus monkeys. The carbinols were long-lived metabolites in the plasma of dogs and accumulated in the plasma compartment with time. Furthermore, the carbinol metabolites were found to concentrate in the brain tissues of treated dogs. Although the precise mechanism for this marked species difference in chronic toxicity is not known, the metabolic differences responsible for the presence of the carbinol metabolites at high concentrations in the plasma and brain over time may play a role in the toxicity observed in the dog. © 1987 Society of Toxicology.

Nabilone, a crystalline compound formulated as a polyvinylpyrrolidone (PVP) codispersion (Cesamet, Eli Lilly and Co.), is a new synthetic 9-ketocannabinoid that has been developed as an orally effective drug for the treatment of nausea and vomiting induced by antineoplastic agents (Herman et al., 1977, 1979; Einhorn et al., 1981; Ward and Holmes, 1985). The present investigation was conducted to evaluate and to compare the interspecies acute, subchronic, and chronic toxicity of nabilone.

The toxicity of Δ^2-tetrahydrocannabinol (THC) and other naturally occurring cannabinoids has been studied extensively in a variety of species and under different treatment durations (Dewey et al., 1972; Rosenkrantz et al., 1975; Rosenkrantz and Braude, 1975; Thompson et al., 1973a,b, 1974). However, relatively little is known of the toxicologic
profiles of synthetic cannabinoids. The results to be presented show that a marked species difference in the chronic toxicity of nabilone exists between the dog and rhesus monkey and that this contrast may be related to a striking difference in the metabolism of this synthetic cannabinoid by the two species.

METHODS

Materials. Nabilone, (±)-trans-3-(1,1-dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-9H-dibenzo[b,d]pyran-9-one, was prepared as a codispersion with polyvinylpyrrolidone in approximately a 1:9 ratio. Nabilone is a racemic mixture consisting of equal parts of the enantiomeric RR and SS ketones, each having a fixed trans configuration at the 6a, 10a ring junction (Archer et al., 1977).

Acute toxicity. The acute toxicity of nabilone-PVP (oral route) was examined in ICR mice and Wistar rats (10 M, 10 F/dose), in beagle dogs (2M, 2 F/dose), and in rhesus monkeys (2 M, 2 F/dose). The dose was prepared as a suspension in a 10% (w/v) acacia vehicle for each species except the dog (capsules). The animals were observed for 14 days. Cats (1 M, 1 F) were given single 1 mg/kg oral dose of nabilone in a 1% Tween-80 vehicle and observed 7 days. The acute toxicity of nabilone by the intravenous route was examined in beagle dogs (1 M, 1 F) given single 1 mg/kg doses of nabilone dissolved in ethanol; the dogs were observed for 7 days.

Subchronic toxicity. A 3-month study was conducted in Fischer-344 rats (15 M, 15 F/dose) fed diets containing nabilone-PVP at concentrations (w/w) of 0, 0.00156, 0.00625, 0.025, or 0.1 mg/kg, respectively, which provided time-weighted average daily nabilone doses of 0, 1, 5, 19, or 93 mg/kg, respectively. The control rats were fed the diet only (Purina Certified Rat Diet No. 5002). Tests in beagle dogs (2 M, 2 F) consisted of a 2-week study in which the animals were given single, daily intravenous doses of 0.4 mg of nabilone/kg (ethanol vehicle), and a 3-month study in which dogs (2 M, 2 F) were given single daily oral doses in capsules of nabilone-PVP equivalent to 0, 0.25, 0.5, or 1.0 mg of nabilone/kg. The experimental parameters examined in these studies are summarized in Table 1. Serum clinical chemistry measurements were performed on a SMA 6/60 Autoanalyzer or a SMA 12/60 Autoanalyzer. All animals were caged individually and provided with tap water and daily food rations (Wayne Laboratory Dog Diet, Allied Mills Co., Castleton, IN).

Ophthalmoscopic and biomicroscopic examinations of the eyes were conducted prior to the test and at about 4 months; an additional ophthalmoscopic examination was performed at the end of the study. Hematologic, urinalysis, and serum clinical chemistry measurements (see Table 1) were performed on samples obtained prior to the test, at the end of the first, second, and fourth weeks, and at monthly intervals thereafter.

Rectal temperatures were measured 6 hr after the first dose and prior to dosing on Days 2, 3, 4, and 5. Tear secretion rates were measured on Days 7, 14, 21, 92, and 129 using the Schirmer test (Schirmer Tear Test, sterile strips, SMP Division of Cooper Laboratories, PR).

Plasma concentrations of nabilone and total carbinol metabolites of nabilone1 formed by the reduction of the 9-keto group (Billings et al., 1980) were monitored during the course of the study. The analyses were made by the GC/MS procedure of Sullivan et al. (1978) on plasma samples taken at 1, 2, 3, 4, 6, and 24 hr after dosing on Days 1 and 168. Additional measurements were made on samples taken 1 and/or 24 hr after dosing on Days 31, 93, 153, 168, 175, 183, 189, and 204. At three times during the study, plasma and brain tissue (i.e., cerebral cortex, cerebellum, amygdala, hippocampus, and hypothalamus from one sagittal half) were collected from treated dogs at the time of death to analyze the content of nabilone and its carbinol metabolites.

The study, originally designed for a 1-year period, was terminated after approximately 7 months (204 test days) because of high mortality among the treated animals. Since convulsions often preceded death in the treated dogs that died during the first 5 months, a decision was made to kill all treated animals which convulsed or became moribund from Day 163 to the termination of the test to avoid the potential loss of clinical information (e.g., clinical chemistry, hematology, and pathology findings) due to postmortem changes. Additional parameters were examined in an attempt to elucidate the mechanism of toxicity. On Day 197, approximately 4 to 6 hr

1 In principle, each antipode of nabilone can yield an axial and an equatorial carbinol metabolite upon reduction of the 9-keto group (viz., SSR and SSS carbinols from SS-nabilone, and RRS and RRR carbinols from RR-nabilone). The chromatographic techniques employed did not permit the separation of the axial carbinol mixtures (i.e., SSR vs RRS-enantiomers) or the equatorial carbinol mixtures (i.e., SSS vs RRR-enantiomers). Hence, in the text the axial and equatorial carbinol isomers will be designated as RRS(SSR) and SSS(RRR), respectively. Total carbinol will refer to the sum of the four diastereomeric carbinols.
after dosing, one male and one female from each group were individually placed in an isolation room for observation while subjected to successive 2-min periods of sound at the following frequency-intensity combinations: 2 kHz, 105 dB; 4 kHz, 112 dB; 8 kHz, 108 dB; and 10 kHz, 87 dB. Photic stimulation (in darkened room) was conducted at increasing pulse frequencies (110 to 25,000 flashes/min) using a Strobatac type 153A stroboscope (General Electric Co., West Concord, MA). Total CO₂ in serum taken on Day 199 was analyzed on a Beckman chloride-CO₂ analyzer (Beckman Instrument Co., Fullerton, CA). Serum taken on Day 199 was also analyzed for sodium, potassium, calcium, and magnesium content by atomic absorption spectrometry. Serum phosphate was determined by the method of Rand et al. (1975).

All animals were necropsied following death. Organ weights (see Table 1) were measured and the following tissues were collected for gross and microscopic pathologic examinations: kidney, liver, heart, lung, spleen, thymus, lymph node, salivary gland, pancreas, stomach, intestine, ovary, uterus, adrenal, thyroid, testis, prostate, skin, mammary gland, skeletal muscle, urinary bladder, brain, pituitary, bone, bone marrow, eye, and gallbladder.

One-year toxicity in monkeys. Rhesus monkeys (Charles Rivers Breeding Laboratories, North Wilmington, ME), in the initial weight ranges of 3.1 to 6.3 kg for females and 3.3 to 6.6 kg for males, were randomly assigned to one of five groups, each consisting of three males and three females.

Nabilone-PVP was suspended in a vehicle which consisted of 10% w/v acacia in distilled water and given by nasogastric intubation (dose volume of 2.0 ml/kg); control animals were given the vehicle only. The monkeys in the treated groups received single daily doses equal to 0.1, 0.5, or 2.0 mg of nabilone/kg. A fifth group of monkeys also received 2.0 mg of nabilone/kg as described above but were dosed on an intermittent basis, i.e., 2-week periods of daily dosing each followed by an interval of 2 weeks without treatment. This periodic regimen was included to provide an approximation to the maximum anticipated duration of clinical treatment.

Daily food rations (Purina Certified Lab Monkey Chow No. 5048) were provided for consumption ad libitum. Ophthalmoscopic examinations were conducted prior to the test and at the termination of the study. Tear secretion rates were measured pretest and on Days 2, 6, 14, 34, 92, 184, and 360 using the Schirmer test.

Hematologic, clinical chemistry, and urinalysis measurements (Table 1) were made on samples taken prior to the start of the study and at monthly intervals thereafter. At various times during the course of the study, blood samples were taken to quantify the plasma levels of nabilone and its carbinol metabolites by the GC/MS procedure described above. All blood samples were drawn prior to dosing on each occasion.

All monkeys were necropsied following death. Organ weights (see Table 1) were obtained, and pathologic evaluation of tissues was conducted as described above for the chronic study in dogs.

**RESULTS**

**Acute Toxicity**

The LD₅₀ for nabilone-PVP given orally to male and female mice was greater than
1000 mg of nabilone/kg. Transient signs of toxicity seen at doses of 500 and 1000 mg/kg in both sexes consisted of limb weakness, hypoactivity, tremors, and extensor rigidity. Poor grooming and alopecia occurred in males and Straub tails were observed in females. All rats given single oral doses of nabilone-PVP equal to 1000 mg of nabilone/kg survived. Transient signs of toxicity were similar in both sexes and consisted of hypoactivity, ataxia, limb weakness, poor grooming, extensor rigidity, and diarrhea or soft stools; tremors were seen only in females.

Cats given single 1 mg/kg oral doses of nabilone had mild ataxia and a slight degree of mydriasis which began 1 hr after dosing and persisted for about 3 hr. One cat vomited but showed no other untoward effects.

Dogs given the nabilone-PVP codispersion at a dose equal to 1 mg of nabilone/kg became ataxic approximately 2 hr later, but all animals were normal by the following day.

Dogs given nabilone-PVP orally at a dose equal to 5 mg of nabilone/kg survived but showed severe and prolonged effects. Mydriasis, slow pupillary response to light, ataxia, and sedation occurred within the first 1 to 3 hr after dosing. Other early effects seen occasionally in some of the dogs given 5 mg/kg were myoclonus, retching, emesis, salivation, dry mouth, hyperirritability to sound, and mild tonic seizures (one dog). All the dogs were still ataxic, lethargic, and had slow pupillary responses at 24 hr but recovered gradually during the second through fourth days of the test. Dogs given single intravenous doses of nabilone equal to 1 mg/kg promptly became ataxic for about 10 min and subsequently lost consciousness for about 48 hr. Upon regaining consciousness, the animals were anorectic for about 2 days but were normal by the fifth day after dosing.

Rhesus monkeys had a higher tolerance than dogs to the CNS depressant effects induced by acute oral nabilone-PVP treatment. One of two monkeys given a dose equal to 5 mg/kg of nabilone (nasogastric route) had slight ataxia 1.5 hr after dosing but was normal by the end of the first 3-hr period. All monkeys given 10 mg/kg became hypoactive and sedated within 15 to 30 min and remained in this condition during the first day; anorexia was evident. The animals appeared to be normal by the second day and showed no further effects.

**Subchronic Toxicity in Rats**

Fischer-344 rats tolerated dietary mixtures of nabilone-PVP which provided time-weighted average daily nabilone doses of 1, 5, 19, or 93 mg/kg. All animals survived. Rats in the lowest dose group showed no effects of treatment except for marginal reductions in body weight and food intake in males. Rats in the upper three dose groups had slight-to-moderate decreases (23 to 35% below controls) in final body weight gain, which correlated with dose-related decreases in food consumption except for animals in the highest dose group which had food intake equal to or greater than that of controls during the test.

Signs of hyperactivity began to appear during the first 3 weeks in all but the lowest dose group. This condition persisted in rats in the 5 mg/kg group, but the hyperactivity diminished in rats in the upper two dose groups, and the animals subsequently became hypoactive during the latter half of the test. All animals in the top three dose levels also exhibited hyperirritability to touch. The rectal temperatures of rats in the two highest dose groups were increased slightly (1°C above controls) when measured at the end of the first week of treatment; this correlated with the onset of the hyperactivity. There were no treatment-related pathologic changes or adverse effects on the clinical chemistry, hematologic, or organ weight parameters examined.

**Subchronic Toxicity in Dogs**

All dogs given daily intravenous doses of 0.4 mg/kg for 14 or 15 days survived. Overt
effects observed after dosing consisted of hyperirritability to touch, sedation, decreased respiration, fine tremors, ataxia, and anorexia. Only hyperirritability to touch and occasional episodes of ataxia persisted after the first week.

Slight-to-moderate increases in BUN and serum aspartate transaminase activity occurred at the end of the first week. Hematological changes were limited to an elevation in the myeloid-to-erythroid ratios in bone marrow and reflected a response to localized injury at the injection site. Urinalysis and organ weight parameters were unaffected by treatment, and pathologic changes were limited to thrombophlebitis and inflammation at the injection site.

All dogs given daily oral doses of nabilone-PVP equal to 0.25, 0.5, or 1.0 mg of nabilone/kg for 3 months survived. Transient episodes of ataxia (middle and highest dose) and anorexia (highest dose) occurred during the first week of treatment. There were no treatment-related adverse effects on any of the clinical chemistry, hematologic, organ weight, or pathology parameters examined.

Chronic Toxicity in Dogs

Relative to the subchronic studies, the chronic treatment of dogs produced markedly different results. Deaths occurred at all dose levels among the treated animals. Of the eight animals in each group, 2, 6, or 7 died or were killed in the groups given daily doses of 0.5, 1.0, or 2.0 mg/kg, respectively, by the end of the study (Day 205). The duration of time prior to death during the first 3 to 4 months appeared to reflect an inverse relationship to dose (Table 2).

One or more convulsive episodes were often observed to occur at some time during the 24-hr period prior to death, although there was considerable variability with respect to the time between the onset of the seizures and the time of death. The convulsions generally occurred without prior overt signs of morbidity and/or other indications of gradual deterioration in the physical condition of the animals. One male (2.0 mg/kg) was killed after being found in a moribund state with a prolapsed ileum (Day 76); the exact relationship of this condition to treatment could be not determined.

Several transient overt effects were noted in some or all of the dose groups during the first month of the study and consisted of (1) a slight decrease in mean rectal temperatures (−1.3 to −1.7°C) at all doses initially (i.e., at 6 hr after the first dose) but persisting for an additional 48 hr in some high dose animals; (2) anorexia in most treated dogs, and ataxia and ptosis in all treated dogs during the initial week; (3) hypoactivity at all doses for the first 2 to 4 weeks; and (4) slight body weight reductions primarily at the upper two doses during the initial month.

Treatment-related overt effects which persisted beyond the first 4 weeks of treatment were limited to mydriasis in all dogs for approximately 4½ months and reduction in tear secretion. The decreases in tear flow at the end of the first and second weeks were generally moderate to severe in a number of dogs from the upper two dose groups. In a few of the animals, tear secretion ceased completely and was probably responsible for the development in one dog (1.0 mg/kg) of a moderate-to-severe mucopurulent conjunctivitis with ulceration of the right cornea and prolapse of the iris. Conjunctival hyperemia was observed in approximately half of the total number of animals in each dose group; the onset of this condition coincided with tear flow reduction and persisted for up to 3 weeks in affected dogs from the upper two dose groups. After daily treatment of eyes with an artificial tear solution (Tears Naturale, Alcon Laboratories, Inc., Fort Worth, TX) during the second and third weeks, the tear flow rate in most dogs improved and showed considerable recovery by the fourth month.
When exposed to sound or photic stimuli on Day 197, none of the control or treated dogs developed seizures. The treated dogs often showed some signs of increased activity (e.g., panting, trembling, defecation, or increased movement) at the lower sound frequencies but became calm as the frequency was increased.

The absorption of nabilone in dogs was relatively rapid after oral administration. Plasma levels of nabilone measured in samples taken after the first dose were highest at 1 hour (Fig. 1) and declined steadily during the next 5 hr; the subsequent rate of plasma disappearance during the 6- to 24-hr period was slow.

Nabilone was rapidly and extensively converted by reduction to the carbinol metabolites in the dog. The carbinols were long-lived in plasma and attained peak plasma concentrations (by 3 to 6 hr) which were about four to five times the respective peak levels of nabilone at each dose level. There were no consistent sex differences in mean peak nabilone levels across dose groups.

Plasma concentrations of nabilone and carbinol metabolites were monitored at various times during the test in samples taken 1 hr (e.g., peak) and/or 24 hr (e.g., trough) after dosing (see Figs. 2 and 3, respectively). Only a slight accumulation of nabilone in the plasma compartment occurred at some point between Day 1 and 168 as evidenced by small increases in the 1- and 24-hr plasma levels as well as area under the plasma concentration-vs-time curve (AUC) obtained at the latter point (data not shown). No further increases were evident after Day 168.

Considerable increases relative to Day 1 values were evident in the 1- and 24-hr plasma levels of total carbinol metabolites taken at later times during the treatment period. Comparisons of plasma AUC values on Day 168 (data not shown) with initial values (Day 1) demonstrated that AUC values for the carbinols had about doubled. These data indicated that the carbinol metabolites had accumulated in the plasma compartment at sometime during this time interval. There were considerable fluctuations in the 1- and 24-hr carbinol levels after Day 168, but the mean values generally tended to decline by the end of the study.

Analyses of plasma and brain tissue concentrations in samples collected at the time of death in three treated animals (Table 3) showed that the level of carbinol metabolites in each of the areas of the brain examined was markedly higher than that in plasma. The SSS(RRR)-carbinol metabolites were the major isomers present in plasma and in the brain tissues. The RSS(SSR) isomers of the carbinol metabolites were found, in some instances, in cortex, cerebellum, or hypothala-
FIG. 1. Plasma concentrations of nabilone and the carbinol metabolites of nabilone in dogs after the first oral dose of nabilone-PVP (Day 1). Each value is expressed as the mean + SE (vertical lines) for N = 8. ●, 0.5 mg/kg; △, 1.0 mg/kg; ■, 2.0 mg/kg.

mus but were always at much lower levels than the SSS/(RRR) isomers. In two of the three dogs examined (Table 3), nabilone was found in either the cerebral cortex or cerebellum at concentrations considerably higher than that found in plasma.

Inspection of the available plasma carbinol data showed that there was a poor correlation between the time of death and the magnitude of the 24-hr carbinol metabolite levels in the last plasma samples obtained before convulsions and death. In a few instances, plasma samples were obtained shortly after convulsions occurred in dogs that subsequently died or were sacrificed in moribund condition (Table 4). Analyses of postseizure plasma samples from dogs 143002 and 142672 (1.0 mg/kg) showed considerable differences in concentrations of the carbinol metabolites despite the fact that both dogs convulsed and were sacrificed on the same day. Plasma samples obtained from dog 141943 (2.0 mg/kg) soon after convulsions (the animal died shortly thereafter) contained a carbinol metabolite level which was considerably below those found in other animals which survived.

Nabilone treatment produced no marked effects on any of the serum clinical chemistry or hematologic parameters examined in dogs. In general, the changes observed in these parameters occurred either during the initial phase of treatment when overt toxicity (e.g., ataxia, depressed activity, and anorexia) occurred or near the time of death. The temporal pattern and relatively low magnitude of the changes indicated that they were likely to be secondary either to the disturbed physiologic state associated with CNS depression or to the onset of agonal changes near the time of death. There were no toxicologically significant effects on the urinalysis parameters or on organ weights.

Although deaths were frequently associated with convulsions, no treatment-related
Fig. 2. Plasma concentrations of nabilone and carbinol metabolites in dogs during the course of chronic daily oral treatment with nabilone-PVP. The blood samples were taken 1 hr after dosing on each occasion. Each value is expressed as the mean + SE (vertical lines). Solid lines, nabilone; dashed lines, carbinol metabolites; ●, 0.5 mg/kg; △, 1.0 mg/kg; ■, 2.0 mg/kg.

Fig. 3. Plasma concentrations of nabilone and carbinol metabolites in dogs during the course of chronic daily oral treatment with nabilone-PVP. The blood samples were taken 24 hr after dosing on each occasion. Each value is expressed as the mean + SE (vertical lines). Solid lines, nabilone; dashed lines, carbinol metabolites; ●, 0.5 mg/kg; △, 1.0 mg/kg; ■, 2.0 mg/kg.
TABLE 3

PLASMA AND BRAIN CONCENTRATIONS OF NABILONE AND CARBINOL METABOLITES IN DOGS AFTER CHRONIC ORAL TREATMENT WITH NABILONE-PVP

<table>
<thead>
<tr>
<th>Daily dose (mg/kg)</th>
<th>Sex</th>
<th>Days on test</th>
<th>Plasma concentration (ng/ml)</th>
<th>Cerebral cortex</th>
<th>Cerebellum</th>
<th>Amygdaloid</th>
<th>Hippocampus</th>
<th>Hypothalamus</th>
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</thead>
<tbody>
<tr>
<td>1.0</td>
<td>M</td>
<td>175&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Nabilone 14.4</td>
<td>336</td>
<td>176</td>
<td>64</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>SSS(RRR)-Carbinol Trace</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RRS(SSR)-Carbinol Trace</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>F</td>
<td>204&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Nabilone 5.9</td>
<td>217</td>
<td>233</td>
<td>63.0</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SSS(RRR)-Carbinol 55.7</td>
<td>218</td>
<td>186</td>
<td>246</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RRS(SSR)-Carbinol Trace</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>F</td>
<td>204&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Nabilone 8.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SSS(RRR)-Carbinol 98.4</td>
<td>441</td>
<td>393</td>
<td>326</td>
<td>390</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RRS(SSR)-Carbinol Trace</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Dog killed in moribund condition 7 hr after dose.
<sup>b</sup> Dog killed at termination of study (24 hr after last dose).
<sup>c</sup> Below detectable limits (20 ng/g).

Chronic Toxicity in Monkeys

In contrast to the results obtained from the chronic test in dogs, the toxicity of nabilone in monkeys was low. There were no treatment-related deaths during the 1-year test period.

Overt effects of treatment generally occurred only on the first 2 days of the test at doses above 0.1 mg/kg and consisted of depressed activity (1–2 hr) followed by seda-

TABLE 4

CONCENTRATIONS OF NABILONE AND TOTAL CARBINOL METABOLITES OF NABILONE IN PLASMA SAMPLES TAKEN NEAR THE TIME OF DEATH

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Sex</th>
<th>Nabilone dose (mg/kg/day)</th>
<th>Days on test</th>
<th>Plasma concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nabilone</td>
</tr>
<tr>
<td>143002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>M</td>
<td>1.0</td>
<td>183</td>
<td>6.3</td>
</tr>
<tr>
<td>142672&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F</td>
<td>1.0</td>
<td>183</td>
<td>7.1</td>
</tr>
<tr>
<td>141943&lt;sup&gt;b&lt;/sup&gt;</td>
<td>F</td>
<td>2.0</td>
<td>153</td>
<td>10.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Blood samples were taken just after convulsions; the animals were then killed.
<sup>b</sup> Taken in proximity to time of convulsions; dog found dead later.
tion. Emesis or ataxia occurred in single animals at the highest dose on the first day. A few animals in the 2 mg/kg group were anorectic for several days during the first 5-day period. Only one male (2.0 mg/kg) continued to periodically show signs of anorexia (with slight-to-moderate weight loss) during the first month of treatment. Body weights were otherwise unaffected by treatment. In general, tolerance to the above effects developed rapidly; even monkeys given 2.0 mg/kg on an intermittent schedule did not reexhibit these effects when rechallenged on the second or subsequent periods of treatment.

Eye examinations showed no changes related to treatment. Tear flow rates of treated monkeys were unaffected.

The 24-hr plasma nabilone concentrations (Fig. 4) increased with time at all doses and reached maxima on Day 71 or 99; the plasma levels subsequently showed a declining trend. This pattern was indicative of a transient accumulation of the parent compounds in the plasma compartment. Consistent with this conclusion was the observation that the highest 24-hr nabilone level (Day 99) attained by monkeys given uninterrupted daily doses of 2.0 mg/kg was about three times that attained by monkeys given the same daily dose but on an intermittent schedule (i.e., on alternating 2-week periods). The highest 24-hr nabilone concentration attained by the latter group was actually closer in value to the level of the groups given uninterrupted daily doses of 0.1 or 0.5 mg/kg.

Total carbinol metabolite levels (Fig. 4B) were highest in 24-hr plasma samples taken near the midpoint of the test (Day 211), but the plasma concentrations appeared to be relatively constant during most of the tests. In contrast to the high carbinol-to-nabilone plasma concentration ratio found in nabilone-treated dogs (see Figs. 2 and 3), the carbinol levels in 24-hr plasma of monkeys were actually lower than that of nabilone (Figs. 4A and 4B). During the test, the mean 24-hr carbinol metabolite levels in monkeys in the two lowest dose groups were generally near or below quantifiable limits (i.e., <0.4 ng/ml).

There was no evidence of substantive treatment-related changes in the clinical chemistry, hematologic, urinalysis, or organ weight parameters examined. There were no pathologic lesion in monkeys attributed to chronic nabilone treatment.

**DISCUSSION**

The present investigations were conducted to evaluate and compare the interspecies toxicity of nabilone in laboratory animals. Rodents had a high tolerance to the acute toxicity by the oral route; the large oral LD50 values in rats reported here for nabilone are characteristic of the high LD50 values reported in this species for THC (Thompson et al., 1973b). Like THC, the primary form of acute toxicity observed after nabiline was given orally to rodent and nonrodent species was CNS depression. Among the nonrodent species tested, monkeys had a substantially higher tolerance than dogs to the depressant effects induced by nabilone-PVP.

In subchronic tests, rats tolerated approximate total daily oral doses of up to 93 mg/kg for 3 months with no deaths. Nabilone produced weight reduction, overt behavioral changes, and transient hypothermia characteristic of those produced by THC (Thompson et al., 1973a,b). No other evidence of systemic toxicity was observed in rats. Dogs given daily oral doses of up to 1.0 mg/kg for 3 months showed no effects other than transient episodes of ataxia and anorexia.

The chronic toxicity seen in nabilone-treated dogs which led to convulsions and death was latent and indicative of a process involving cumulative toxicity. The high mor-
A. Nabilone

B. Carbinol Metabolites

Fig. 4. Twenty-four-hour plasma concentrations of (A) nabilone or (B) carbinol metabolites in rhesus monkeys during chronic nabilone-PVP treatment (po). Each value is expressed as the mean ($N = 6$). ●, 0.1 mg/kg; △, 0.5 mg/kg; ■, 2.0 mg/kg; □, 2.0 mg/kg (intermittent).

tality encountered in the chronic test was not anticipated from the relatively low toxicity seen in the 3-month studies conducted in rats and dogs, particularly in view of the fact that the highest dose in the 3-month dog was selected for the chronic study. In contrast to the dog, rhesus monkeys tolerated similar oral doses of nabilone for 1 year, showing only a low level of toxicity.

Although comparable chronic oral toxicity studies for other cannabinoids are not known to have been conducted in dogs, chronic studies in rats indicate that cannabinoid-induced cumulative toxicity is not unique to nabilone. Chronic toxicity studies in rats reported by Rosenkrantz et al. (1975) and by Rosenkrantz and Braude (1975) have shown that the administration of daily oral doses of THC (2, 10, or 50 mg/kg) for a 6-month period induced cumulative lethal toxicity. These investigators found that a biphasic pattern of neurotoxicity occurred which was characterized by an initial period (ca. 1 to 2 weeks) of CNS depression to which tolerance developed, followed by a much longer period in which CNS-stimulatory effects (e.g., hyperactivity, hypersensitivity, fighting behavior) gradually heightened. Eventually, tremors and clonic convulsions were observed after 70 days of THC treatment in 12% of the rats given 10 mg/kg/day and 50% of the rats given 50 mg/kg/day. Peak convulsive activity occurred near the 120th day. Convulsions preceded death in a number of rats in the highest THC dose group, although considerable variability was reported in the time interval between these two events. The cause of death was not established.

In the present investigation, nabilone-treated dogs similarly experienced an initial, transient period of CNS depression to which tolerance rapidly developed. However, the ensuing CNS-stimulatory phase reported to occur in THC-treated rats, and seen here in nabilone-treated rats, was not, in general, evident in the treated dogs. The results of clinical
chemistry, hematologic, urinalysis, and organ weight data, and pathologic evaluations did not elucidate the cause of death in the dogs.

Experiments conducted to determine if nabilone-treated animals were susceptible to seizure induced by audiogenic or photic means showed that neither type of stimuli elicited seizures. Toxicity studies on THC, Δ⁸-THC, and crude marijuana extracts given orally to rats have also demonstrated that convulsions were not induced by auditory or visual stimuli (Thompson et al., 1973a).

In addition to the initial CNS-depressant effect, dogs given chronic nabilone treatment showed transient signs of anorexia, hypothermia, and reduction of tear secretion. These overt effects (most of which were inexplicably undetected in the 3-month nabilone dog study) are known to occur with THC or marijuana in various species. Intermittent anorexia and transient hypothermia have been reported to occur in rhesus monkeys given daily doses of THC (iv or po) for 28 days (Thompson et al., 1973b). Initial, transient periods of hypothermia have also been associated with THC treatment in rats (Rosenkrantz et al., 1975; Thompson et al., 1973a) and in dogs (Thompson et al., 1973a), and with nabilone treatment in rats (Page and West, 1977). Tear flow reduction and conjunctival hyperemia have been reported to occur in humans after smoking marijuana cigarettes (Hepler et al., 1977). In the present study the conjunctival hyperemia and the single incidence of a corneal lesion correlated with the reduction of tear flow in nabilone-treated dogs.

The metabolism of nabilone in the dog (Sullivan et al., 1978, 1987) differs markedly from that described in monkeys (Sullivan et al., 1987) or in man (Rubin et al., 1977). The carbinol metabolites of nabilone formed by the enzymatic reduction of the 9-keto group are minor metabolites in rhesus monkeys and in humans but major biotransformation products in dogs.

Sullivan et al. (1978) studied the metabolism of nabilone in dogs and reported that the carbinol metabolites attained plasma levels which were about four times that of the parent drug after a single oral dose of nabilone. The plasma half-life (elimination phase) of nabilone was estimated to be about 2.5 hr after a single oral dose (200 μg/ml), whereas the half-life for the carbinol metabolites was considerably longer (i.e., 12 to 27 hr).

The present chronic study in dogs shows that the carbinol metabolites of nabilone appear in the plasma at high concentrations relative to those observed in monkeys at comparable doses. The carbinol metabolites were long-lived and accumulated in the plasma compartment of dogs with time. Furthermore, the carbinols, and, in some cases, nabilone per se were found in the brain of treated dogs at concentrations which were substantially higher than those found in plasma.

Although the mechanism for the long-term toxicity observed in dogs given nabilone is not known at present, a species difference in metabolism may be the basis for the marked contrast in chronic toxicity between the dog and rhesus monkey. It is possible that the presence of the carbinol metabolites at high concentrations in the plasma and brain over time may play a role. Subsequent experiments have shown that short-term treatment (data not shown) of dogs with high doses (viz, 2 mg/kg/day, iv) of nabilone or its major SSS-carbinol metabolite for 5 days was not sufficient to produce convulsions despite the fact that the respective 24-hr plasma carbinol metabolite levels during this period were about twice those seen at the highest dose in the chronic dog study.

The carbinols also appear to be major metabolites of nabilone in rats. Billings et al. (1980) reported a high carbinol-to-nabilone plasma concentration ratio 1 hr after a single intravenous dose of nabilone. The present studies, however, do not permit a comparison of the chronic toxicity of nabilone in the dog and rat since the treatment period in the
latter species was limited to 3 months during which the animals tolerated the drug at all doses tested.

The comparative drug metabolism and disposition data on nabilone for the dog, rhesus monkey, and human clearly show the dog to be unique and support the view that the rhesus monkey is a more appropriate test animal than the dog for the preclinical toxicologic evaluation of this drug.

REFERENCES


