An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp

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Abstract

A battery of toxicological studies was conducted on a hemp oil, containing approximately 25% cannabinoids, extracted with supercritical CO_2 from the aerial parts of the *Cannabis sativa* plant. No evidence of genotoxicity was found in a bacterial reverse mutation test (Ames), an in vitro mammalian chromosomal aberration test and an in vivo mouse micronucleus study. A 14-day repeated dose oral toxicity study conducted in Wistar rats at 1000, 2000, and 4000 mg/kg bw/day resulted in effects where a NOAEL could not be concluded. Based on those results, a 90-day repeated dose oral toxicity study was performed in rats using doses of 100, 360, and 720 mg/kg bw/day, followed by a 28-day recovery period (control and high dose group). Significant decreases in body weight and body weight gain, and changes in various organ weights compared to controls were observed. At the end of the recovery period, many of the male and female high-dose satellite groups' results were trending toward normal; thus, the changes appeared reversible. The NOAEL for this hemp oil in Hsd.Han:Wistar rats was considered to be 100 mg/kg bw/day for males and 360 mg/kg bw/day for females.

Introduction

- Cannabinoids are oxygen-containing aromatic hydrocarbon compounds that constitute at least 70 of the estimated 400+ constituents in the *Cannabis sativa* plant .[1–3]
- Strains of C. sativa that contain very low amounts of delta-9-tetrahydrocannabinol (THC), commonly referred to as hemp, tend to contain greater amounts of the lesser known cannabinoids: canna-bichromene (CBC), cannabielsoin (CBE), and cannabidiol (CBD).[2,4]
- CBD is non-sedating and non-intoxicating. There is no compelling evidence that CBD undergoes cyclization or bioconversion to THC in humans.[5]

Bacterial Reverse Mutation (Ames) Test

S. typhimurium strains: TA98, TA100, TA1535, and TA1537 and *E. coli* strain: WP2 *uvrA*.

OECD (471), EC (440/2008 B13/14), EPA (OPPTS 870.5100), ICH (S2(R1)) and GLP compliant.

- No mutagenicity was detected up to the highest level tested (5000 µg/plate).

In Vitro Mammalian Chromosomal Aberration Test

Cells: V79 (Chinese hamster lung) cells.

OECD (473), EC No. 440/2008, EPA (OPPTS 870.5375) and GLP compliant.

- No aberrations, abnormal metaphases, or dose-responses occurred when tested up to cytotoxic concentrations (90 µg/mL with and 5 µg/ mL without metabolic activation).

Test Article

- Manufactured by CV Sciences, Inc. (San Diego, CA) through supercritical CO2 extraction of the oil from hemp grown by certified growers in Europe.
- Soluble in DMSO and sunflower oil
- Hemp oil consists of 61% edible fatty acids, ~25% phytocannabinoids (of which ~96% is CBD and < 1% THC); the remaining 13% includes fatty alkanes, plant sterols, triterpenes and tocopherols.
- Formulated in DMSO for the Ames test, in Dulbecco's modified Eagle's medium for the chromosomal aberration test and in sunflower oil for the mouse micronucleus test and the 90-day repeated-dose oral toxicity study.

In Vivo Mammalian Erythrocyte Micronucleus Test

Male SPF Crl:NMRI BR mice.

OECD (474), EC No. 440/2008, EPA (OPPTS 870.5395) and GLP compliant.

Performed with permission of the laboratory's Institutional Animal Care and Use committee, following principles and guidelines for Care and Use of Laboratory Animals.

- No genotoxicity was detected up to the highest level tested (limit dose of 2000 mg/kg bw).

Ninety-Day Repeated Oral Toxicity Study

Hsd.Han Wistar rats (aged 42–52 days), OECD (408), FDA Redbook IV.C.4.a, and GLP compliant. Performed with permission of the laboratory's Institutional Animal Care and Use committee, following principles and guidelines for Care and Use of Laboratory Animals.

- Ten animals per sex per group for the low- and mid-dose groups and 15 animals/sex/group for the control and high-dose groups were utilized. Five animals each in the control and high-dose groups were utilized as satellite groups which were treated identically to the main groups up to day 90 and then observed during a 28-day recovery period. Dose groups were chosen based on an OECD (407) and FDA Redbook UV.C.3.a-compliant 14-day repeated-dose oral toxicity study in Hsd.Brl.Han Wistar rats in which a NOAEL could not be established because of test article related adverse toxicological effects and histopathological findings. The dose groups used for the 90-day study were 0, 100, 360, and 720 mg/kg bw/day.
- Gavage dosing of the test article (at a dose volume of 5 mL/kg bw, prepared daily prior to administration) was performed daily for 90 consecutive days.
- Evaluated: Mortality, behavior and clinical observations, body weight, food consumption, feed efficiency, ophthalmologic examination, functional observation battery, hematological and clinical chemistry analysis, gross and histopathology and organ weights (absolute and relative). Qualitative and quantitative sperm analyses were conducted on 5 animals from the control and high-dose groups.

- Outcomes:
  - **Mortality:** There were no mortalities during the main study (0, 100, 360, and 720 mg/kg bw/day groups) or in the satellite groups (0 and 720 mg/kg bw/day).
  - **Clinical signs:** Nuzzling up bedding occurred in the mid-dose groups from day 20 or 21 until the end of the treatment period and in the high-dose groups throughout the study. Salivation occurred in seven males and four females in the high-dose groups shortly after administration of the test article during the first four weeks of the study. No clinical signs were observed in the satellite groups during the recovery period; thus, the signs were considered reversible.
  - **Weight/weight gain**: There were test article related significant decreases in body weight and body weight gain in the main group mid- (males only) and high-dose (both sexes) test groups compared to controls along with reduced food consumption in both sexes in the mid- and high-dose groups. The decreases in body weights and food consumption persisted throughout the recovery period for high-dose satellite group males and females.
  - **Blood/serum**: Differences in several laboratory parameters in the main test groups were statistically significantly different compared to controls, but values remained well within or marginal to historical control ranges, were not in a direction of usual concern and/or had no dose relationship, and were without corresponding histopathological findings—thus, they were not considered toxicologically relevant. With the exception of GGT, the following differences were statistically significant during the main study but were not statistically significantly different at the end of the recovery period:
    - Reduced RET values in mid- and high-dose females
    - Elevated GGT in mid- and high-dose males and females (Although the differences between the satellite test group and control remained statistically significant during the recovery period, GGT levels returned to within historical control ranges during that time.)
    - Elevated monocytes in mid- and high-dose females
    - Reduced eosinophils in mid- and high-dose males and in all female test groups
    - Increased red blood cell count in mid- and high-dose females
    - Reduced ALT in high-dose males and reduced AST in mid-dose males
    - Increased alkaline phosphatase in high-dose females
    - Increased cholesterol in high-dose females
    - Increased inorganic phosphorus in mid-dose males and females and high-dose females
    - Increased albumin and total protein in mid- and high-dose males
  - **Organ weights:** Enlarged and pale adrenal glands were observed in 5/10 males and 7/10 females in the high-dose group. Mottled surface of the kidneys was observed in a few individual animals (1 male and 1 female in the low-dose group and 1 female in the mid-dose group). Several absolute and relative organ weights were statistically significantly different than controls during the main study and recovery period (see table). Of those, the changes in fasted body weight and in the absolute and/or relative weights of the liver and adrenal glands in both male and female animals of the mid- and high-dose groups were considered test article related due to the associated changes in GGT in animals of both of those groups as well as the pale and enlarged adrenal glands observed macroscopically.
  - **Male reproductive evaluation:** Similar total sperm counts, sperm morphology, and percentage of motile and immotile sperm cells were observed in the control and high-dose group males at the end of the main study. Thus, no sperm examinations were conducted on the recovery groups.
  - **Histopathology:** Increased diffuse cytoplasmic vacuolation of the adrenal cortical cells was present in the main group high-dose males and females. These lesions were not found in the high-dose satellite groups at the conclusion of the recovery period. Findings not considered toxicologically relevant included:
    - Slight alveolar emphysema and alveolar histiocytosis, which are considered consequences of exsanguination and age of the animal, respectively.
    - Renal focal fibrosis, cysts, and mineral deposits occurred in individual low-dose group animals.
    - Dilation of the uterine horns is considered a a common neurohormonal phenomenon in connection with the proestrus phase of the sexual cycle and occurred with greater frequency in the main and recovery control groups compared to respective high-dose groups.

Summary of Selected Significant Findings in the 90-Day Repeated Oral Toxicity Study

	Clinical Chemistry	Absolute Body and Organ Weights			Organ:Body Weight		Organ:Brain Weight
Group (mg/kg bw/day)	GGT	Body Weight	Liver weight	Adrenals	Liver	Adrenals	Liver
Male (Main groups n=10)							
Control	1.21 ± 0.23	383.3 ± 27.86	9.65 ± 0.95	0.068 ± 0.008	2.515 ± 0.098	0.018 ± 0.002	468.24 ± 40.18
100	1.27 ± 0.36	374.1 ± 42.85	9.70 ± 1.04	0.072 ± 0.008	2.595 ± 0.081	0.019 ± 0.003	479.69 ± 46.21
360	2.11 ± 0.67**	345.7 ± 18.90**	11.05 ± 0.79**	0.080 ± 0.012*	3.196 ± 0.147**	0.023 ± 0.004**	552.65 ± 49.26**
720	3.44 ± 1.06**	329.4 ± 18.86**	13.50 ± 1.37**	0.080 ± 0.012*	4.093 ± 0.228**	0.024 ± 0.004**	670.06 ± 90.43**
Historical Range[a]	0.0–2.2	344–488	7.95–14.14	0.047–0.097	1.916–3.108	0.012–0.023	371.50–660.20
Male (Recovery group n=5)							
Control	0.98 ± 0.18	434.0 ± 16.08	10.65 ± 0.44	0.071 ± 0.007	2.454 ± 0.089	0.017 ± 0.002	513.36 ± 19.85
720	1.48 ± 0.16**	373.2 ± 19.25**	8.93 ± 0.81**	0.063 ± 0.003*	2.392 ± 0.158	0.017 ± 0.001	435.79 ± 31.87**
Female (Main groups n=10)							
Control	1.54 ± 0.29	239.0 ± 18.90	6.42 ± 0.40	0.083 ± 0.013	2.693 ± 0.174	0.0348 ± 0.0043	326.63 ± 20.94
100	1.35 ± 0.47	234.6 ± 15.97	6.70 ± 0.56	0.084 ± 0.014	2.854 ± 0.133	0.0357 ± 0.0051	357.88 ± 44.84
360	3.11 ± 0.74**	224.3 ± 13.14*	7.40 ± 0.60**	0.093 ± 0.013	3.298 ± 0.144**	0.0413 ± 0.0054*	391.14 ± 50.90**
720	7.41 ± 1.72**	213.4 ± 11.75**	9.21 ± 0.54**	0.100 ± 0.012*	4.322 ± 0.281**	0.0469 ± 0.0068**	490.87 ± 33.83**
Historical Range[a]	0.0–2.9	206–285	5.30–7.97	0.063–0.113	2.172–3.214	0.026–0.044	276.04–412.95
Female (Recovery group n=5)							
Control	1.06 ± 0.15	248.4 ± 8.73	6.63 ± 0.45	0.071 ± 0.009	2.671 ± 0.167	0.0285 ± 0.0031	325.87 ± 21.65
720	1.96 ± 0.46**	217.2 ± 9.58**	5.86 ± 0.21**	0.075 ± 0.005	2.700 ± 0.064	0.0345 ± 0.0027*	305.45 ± 14.34

Data represent the mean values and the standard deviation.
*P < 0.05 and **P < 0.01, statistical significances were determined with Duncan's multiple range test or with Mann-Whitney U-test vs. control
[a]minimum and maximum levels reported as the range of historical control values

Conclusions

- No evidence of in vitro mutagenicity or clastogenicity, nor in vivo genotoxicity was detected in the Ames, chromosomal aberration and in vivo mouse micronucleus tests.
- No target organs or treatment-related toxicological effects were identified. The NOAEL was considered to be 100 mg/kg bw/day for male and 360 mg/kg bw/day for female Hsd.Han:Wistar rats.

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