Animal studies

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Subchronic effects

158. Shara *et al*., (2003) evaluated the dose- and time-dependent effects of HCA-SX in male and female Sprague-Dawley rats on body weight, hepatic and testicular lipid peroxidation, DNA fragmentation, liver and testis weight, expressed as such and as a percentage of body weight and brain weight, and histological changes over a period of 90 days. An animal research protocol (ARC# 0598) was obtained from Creighton University Medical Center. HCA-SX "a natural, highly water-soluble", calcium-potassium salt of 60% HCA extract from *G. cambogia* – commercially known as SuperCitriMax HCA-600 SXS was dissolved in water and administered by gavage at 0, 0.2, 2.0 and 5% of feed intake. Control animals received the vehicle (water). Food and water consumption were measured 2-3 times weekly. Mortality/morbidity was assessed once daily throughout the study period. Clinical signs were evaluated once to twice daily. Body weights were recorded on day 1, twice weekly thereafter and before necropsy. Animals were sacrificed on days 30, 60 and 90 of treatment and the target organs were either processed immediately, preserved in 10% buffered formalin for histopathology, or stored at -80ºC. The number of animals per dose group was not explicitly stated by the authors; however, it was detailed that all result values were reported as a mean ± standard deviation from 5-7 samples.

159. By day 90, feed intake was reduced by 13.7, 26.7 and 25.6% in male rats following supplementation of HCA-SX at the aforementioned doses (0 (water), 0.2, 2.0 and 5% of feed intake), respectively, when compared to their corresponding controls. In females, the values were 16.3, 19.6 and 22.8%, respectively. Regarding changes in body weight, ~11.2, 12.4 and 15.8% reduction in body weight in male rats following supplementation at the tested dose levels, respectively. For females these values were ~11.1, 18.1 and 13.0%, respectively. These were considered as significant by the authors. Regarding changes in testicular weight (expressed as a percentage of body weight and brain

weight), a small but not statistically significant increase in testis weight with increasing age was observed in control animals. The testis weights in the exposed groups were similar to the control animals. Regarding the effects of HCA-SX on hepatic and testicular lipid peroxidation, a time-dependent increase in hepatic lipid peroxidation was generally observed in all samples; however, these were not significant in the HCA-SX exposed groups. A small but non-significant increase in testicular lipid peroxidation was observed in the control group as well as the HCA-SX exposed group. Regarding the effects of HCA-SX on hepatic and testicular DNA fragmentation, the results showed that there were no significant HCA-SX treatment related effects on these parameters (in male rats for the latter) when compared with their respective controls. Histopathological analyses on the liver, brain and testis, revealed that HCA-SX exposure did not cause any morphological alterations in these tissues.

160. The authors were of the opinion that these results indicated that HCA-SX was “safe and efficacious in weight management” under the test conditions.

Reproductive toxicity

161. Deshmukh et al., (2008a) evaluated the effects of a novel calcium/potassium salt of HCA on the reproductive systems of male and female rats, the postnatal maturation and reproductive capacity of their offspring, and possible cumulative effects through multiple generations. The study was performed in compliance with a standard study protocol based on the US FDA Redbook Guidelines for Reproductive Studies IV.C.9.a, and Guidelines for Developmental Toxicity Studies IV.C.9.B., Feed Additive Safety (US FDA, 1993), and the OECD principles of Good Laboratory Practice.

162. The test article, HCA-SX, commercially known as Super CitriMax was mixed with powdered rodent diet to obtain three concentration levels. A small volume of diet premix was prepared, which was then mixed with the remaining portion of the diet in a mechanised ribbon blender for about 20 minutes to obtain the desired homogeneity of the test article concentration in diet. The experimental diets were prepared once a week based upon the results of the stability tests on HCA-SX. The diet preparation procedure was subsequently validated by conducting stability studies and homogeneity on exposed diets.

163. Sprague-Dawley rats (n=30/sex per dose group) were administered feed containing HCA at dose levels of 0, 1,000, 3,000, or 10,000 ppm for 10

weeks prior to mating, during mating, and, for females, through gestation and lactation, across two generations. The control group of animals were fed normal diet. The male and female rats of the F0 generation from each dose group were mated and allowed to deliver normally. At weaning, one male and one female pup from each litter from the control and treatment dose groups were selected for the F1 generation. The selected F1 animals were exposed to HCA-SX for 10 weeks before mating and then they were mated to produce a second generation F2a. During the period of study, animals were examined daily for signs of clinical toxicity and their body weight and feed consumption (g/rat/day) were recorded twice a week. N.B. A discrepancy was noted where the abstract states the latter was performed twice a week recording, whilst the methods section describes one weekly recording. For the parents (F0 and F1) and the offspring (F1 and F2a), reproductive parameters such as fertility and mating, gestation, parturition, litters, lactation, sexual maturity, and development of offspring were assessed. At termination, necropsy and histopathological examinations were performed on all animals.

164. Data on feed consumption by the parental male and female rats of both (F0 and F1) generations during the premating and mating periods, for both sexes, and during gestation and lactation in the case of female rats, did not reveal any “remarkable” treatment-related changes in the average daily feed intake by the male and female rats compared to the respective control groups, across the different dose levels for each of the F0 and F1 generations and also when compared across these two generations. Based on feed intake, the resulting dose of HCA-SX for the highest-dose groups of male and female was calculated as 813 and 1,205 mg/kg/day, respectively, for the F0 generation, while the same was respectively 1,018 and 1,524 mg/kg/day in the case of the F1 generation. The total daily dose of HCA-SX for all groups are provided in Table 3.

Table 3 – Daily dose of HCA-SX (mg/kg bw per day) during the premating period (reproduced from Deshmukh et al., (2008a).

HCA-SX level in diet (ppm)	**F0 Males (mg/kg bw per day**	**F0 Females (mg/kg bw per day**	**F1 Males (mg/kg bw per day**	**F1 Females (mg/kg bw per day**
Control (0)	0	0	0	0

Low (1,000)	80	109	89	135
Mid (3,000)	246	354	268	447
High (10,000)	813	1,205	1,018	1,524

165. Dietary exposure to all animals and offspring at the F0 and F1 generations, did not reveal significant incidence of mortality or abnormal clinical signs. Compared to the respective controls, the HCA treatment groups at all dose levels, did not have different feed consumption or body weight. All deaths and abnormal clinical signs observed in the rats during F0 and F1 generations, such as transient/reversible spells of emaciation, abdominal breathing, respiratory rates, hypoactivity, circling disorder, and lacrimation, were considered to be incidental and not test substance related.

166. The average bodyweight and body weight gains on the parental F0 and F1 generations up to all stages including lactation of female rats of the exposed groups at all doses, did not reveal any significant differences when compared to the respective control groups. Although, a mild but significant lowering of body weight gain in F1 male offspring was noted in the highest dose level after they were weaned, between 3 to 7 weeks of their life. For the duration of the study, the difference in body weights persisted; however, it was not significant, and the percentage gain in body weights between control and exposed groups of male rats was found to be comparable on week 31 (it was slightly higher in the high dose group than the control group). The authors did not count this as an adverse effect while deciding the NOAEL, as it was considered to be a likely effect of the pharmacological activity of HCA-SX. There were other “occasional” instances of group mean values of exposed animals differing from those of the respective control; however, these were not considered of no toxicological significance due to the “small magnitude of variation”.

167. During the gestation period in all females exposed to HCA-SX, no treatment-related adverse effects on reproductive performance in terms of fertility and mating, gestation, parturition, and the litters born were observed. The values of male fertility indices for the exposed groups in the F0 and F1 generations did not differ significantly from those of the controls and also compared well with the historical control data at the test facility. The sperm motility of the F1 parents was lower than the F0 parents (in exposed groups). The

authors did not consider this to be related to HCA-SX exposure, as the lowering was also observed in the concurrent control group of rats.

168. All the gross and microscopic findings of the parental organ weights, necropsy and histopathology were considered to be incidental as the incidence was found to be comparable among the control group and the treatment groups, without any dose-dependent trend.

169. Offspring observations are hereby summarised. The body weights of some of the pups selected as parents for the next generation were recorded at ~4 weeks after they were weaned at the end of their lactation; in male offsprings exposed at the highest dose (10,000 ppm), the group mean body weights were significantly lower than those of the control group; however, this effect was not considered as an adverse effect [was considered as a pharmacological effect]. When compared with their respective controls, data on survival and clinical observations recorded for the offspring of both generations F1 and F2a during the lactation period of 21 days did not reveal any remarkable differences. Nor were there any adverse effects on their litter sizes, sex ratio of litters, live birth indices and the viability of litters, which were calculated on days 4, 7, 14 and 21 of lactation from parental females exposed to HCA-SX at all dose levels. The sexual maturation [age at which there is balanopreputial separation in males and vaginal opening in females] was only measured for the F2a groups. It was observed that exposure to HCA-SX at any of the dose levels did not affect the age of sexual maturity of the offspring belonging to the F2a generation. Exposure of the parental animals of both the F0 and F1 generation to HCA-SX at the tested dose levels had no adverse effect on the physical development of their litters, compared to the respective control groups. The group mean values of absolute and relative weights of the brain, spleen, and thymus of the pups of the F1 and F2a generation did not significantly alter between the control and treatment groups. The study authors considered that all the gross and microscopic pathology findings in this study were accidental as the incidence was found to be comparable among the control group and the treatment groups, without any dose-dependent trend. HCA-SX treatment did not cause any significant histopathological changes in any organ.

170. The authors noted that exposure to HCA-SX did not affect reproductive performance as evaluated by sexual maturity fertility and mating, gestation, parturition, litter properties, lactation, and development of the offspring. Nor did it induce any systemic toxicity in the parental rats and their offspring at the tested concentration levels. Based on the results of this study, the authors determined a

NOAEL to be greater than 10,000 HCA ppm in the diet or equivalent to 1,018 and 1,524 HCA mg/kg bw per day in male and female rats, respectively.

171. Saito et al., (2005) investigated the ability of G. cambogia extract to suppress body fat accumulation in developing male Zucker obese (fa/fa) rats. 6-week-old rats (n=6 per dose group) were fed diets containing HCA powder (41.2 wt%: ratio of free to lactone form was 36.6 to 63.4) at 0, 2, 10.1, 20.1 and 30.2 g HCA/kg diet for 92 or 93 days. All groups had free access to water. Each diet group was pair-fed to the 30.2 g HCA/kg diet group. On the last experimental day, the rats were allowed to consume three-quarters of the food intake of the previous day and were then killed by cardiac puncture. Liver, kidney, spleen, spleen, testis, and epididymal fat pads were excised, washed with isotonic saline and weighed. The liver, spleen and testis were fixed with 10% formalin neutral buffer solution, pH 7.4 and histopathological examinations were performed after haematoxylin-eosin staining. At the highest dose, there was significant suppression of epididymal fat accumulation in developing male Zucker obese rats, compared with the other groups. The higher dose groups (20.1 and 30.2 g HCA/kg diet) caused “potent” testicular atrophy and toxicity, whereas diets containing 10.1 g HCA/kg diet or less did not. The authors derived a NOAEL of 10.1 g HCA/kg diet equivalent to 389 mg HCA/kg BW per day.

172. Burdock *et al*., (2005) performed a “critical review” of the article by Saito *et al*., (2005) (as summarised in paragraph 171) and raised the following comments: (1) the form of HCA and toxicity; (2) experimental study design and results; (3) Zucker rat model and testicular toxicity; and (4) dietary ingredients and testicular toxicity. The form of HCA can vary its toxic profile, dietary supplements containing HCA consist of various salts, including HCA–sodium, –calcium, –potassium, –magnesium, or combinations thereof. Depending on the salt(s) used and the extraction process, the solubility, bioavailability, bioefficacy and lactone content can vary considerably.

173. Limitations on the experimental design were noted: the amount of food received by pair-fed animals was approximately 10% less (compared to ad libitum fed control, which was not included). Burdock *et al*., further state that HCA is known to affect satiety, effectively preventing consumption of food at high levels. Secondly, the investigators reported severe diarrhoea at the highest use levels (1,244 mg HCA/kg/day), which may have further affected the feed intake in this group and other groups (as a result of pair feeding). Thirdly, Saito *et al*., (2005) stated that Zucker obese rats (or models with higher lipogenic properties) may be insensitive to HCA at usual dietary levels; however, a biphasic effect of

HCA on fat accumulation in the liver was noted. The selection of Zucker rat model was thought to be inappropriate since serum concentrations of testosterone in obese male Zucker rat at the age of 2-, 3- and 4-months were lower when compared to lean male Zucker rats. Testes morphology of Zucker rats was also found to be different from lean rats.

174. It was not made clear by Saito *et al*., (2005), whether dietary imbalance or nutritional imbalance (use of high levels of the extract, pair feeding) may have contributed to the observed testicular toxicity at two highest doses of *G. cambogia* extract.

Developmental toxicity

175. Deshmukh *et al*., (2008b) conducted a follow-up study (summarised in paragraphs 161-170) to evaluate maternal toxicity and effects on the developing embryo in Sprague-Dawley rats (effects included death, structural abnormalities, and altered or retarded growth) when exposed to HCA-SX. The study was performed as per the previous protocol in Deshmukh et al., (2008a). A total of 30 males and 30 female pups per dose group (except the 3,000 ppm (mid) dose group where 25 of each sex were available), including the concurrent control group. The animals in this study were selected randomly after weaning from each F2b litter of the F1 generation from the two-generation reproductive toxicity study (as summarised in paragraphs 161-170). Therefore, the rats in the treatment group were exposed directly to HCA-SX via feed, prior to which they had indirect exposure to HCA during lactation. The dietary exposure levels were the same as those employed for the two-generation reproductive toxicity study: 0, 1,000, 3000, or 10,000 ppm. Following mating at maturity, the pregnant rats were observed twice daily for clinical signs of adverse effects, and body weight and feed consumption were recorded. On day 20 of gestation, animals were subjected to a necropsy and caesarean section to examine the uterus, ovaries, and foetuses for assessment of different parameters of pregnancy and embryo-foetal defects.

176. The daily amount of HCA-SX consumed at dietary feed levels of 1,000, 3,000 or 10,000 (ppm) (equivalent to 0.1%, 0.3% and 1.1%) were calculated as 103, 352, or 1,240 mg/kg bw per day, respectively.

177. Comparison indices of sperm-positive females (mating behaviour), maternal deaths during pregnancy, number of pregnant/non-pregnant females, pregnancy rate (%), and females with resorptions (%) were evaluated between

the control group and the HCA-SX exposed groups. At the dose levels administered, no adverse effects were observed. Maternal body weight changes during gestation were recorded for the following periods: days 0, 7, 14 and 20. The group mean values of body weight gain for each period did not show any significant differences between the exposed and control groups. On the 20th day of gestation, a significant decrease (by 13%) in mean body weight of the rats maintained on the highest dose (10,000 ppm) was noted. During the study, no treatment-related clinical effects were observed in any of the groups. However, the following incidences were reported: i) wryneck and a transient period of emaciation were observed in females during the course of the study [was deemed to be not dose related by the authors]; ii) on day 17 of gestation, one pregnant female from the highest dose group died – profuse haemorrhage [by vaginal bleeding] was identified as the probable cause of death. No other mortalities were noted in any of the groups during the course of the study. Observations made on the gravid uteri of females euthanised on day 20 of gestation did not reveal any "remarkable" alterations indicative of adverse effects of HCA-SX on absolute uterus weight, number of: corpora lutea, implantations, live and dead implants, early and late resorptions, and post-implantation losses (%). Observations made on the litters of females euthanised on day 20 of gestation did not reveal any remarkable treatment-related alterations in litter size, number of foetuses, sex ratios and foetal weights. The group mean litter size from the highest-dose group of HCA-SX was significantly lower than the control group (p 0.05). However, these observations deemed to be not of biological significance by the authors as the changes were smaller in magnitude compared to the variation observed in the historical control data.

178. Observations in the foetal groups are summarised. The number of foetuses examined in the control, 1,000, 3,000 and 10,000 ppm dose groups were 226, 227, 158 and 160, respectively. The only "major malformation" was an omphalocele [birth defect, where the intestine or other abdominal organs stick out of the belly button], which was found in two foetuses: one in the control and high-dose groups. This was considered incidental by the authors. Two other "minor malformations" were observed. An incidence of a small haematoma at the tip of the tail in three foetuses: one in the control, low-dose and mid-dose groups. One foetus in the high-dose group was small (runt). No treatment-related significant incidence of soft tissue alterations in foetuses of dams exposed to HCA-SX at the tested doses were noted (n=108, 106, 74, 76 for the control and each HCA-SX dose groups, respectively). Small incidences of "minor anomalies" were observed including, globular heart and unilateral enlargement of the ventricle of the heart, mottled lungs, unilateral displacement of adrenal, and

unilateral hypoplasia of kidney in foetal soft tissues. These were considered as incidental and of no toxicological significance by the authors. The only abnormal finding classified under “major anomalies” was unilateral cerebral hypoplasia observed in one of the foetuses in the low-dose group; this was considered to be incidental by the authors, in light of its isolated incidence (0.94%). There was no incidence of any significant and treatment related skeletal abnormalities in foetuses of dams exposed to HCA-SX. Any abnormalities were considered incidental or of no toxicological significance due to either being a normal variant, minor in nature or the incidence was in isolation and/or comparable to the control dose group.

179. The authors concluded that HCA-SX was not found to be teratogenic in Sprague-Dawley rats at dietary exposure levels of 1,000, 3000, and 10,000 ppm, equivalent to the dose levels of 103, 352, or 1,240 mg/kg/day, respectively. Based on the results of the study, the NOAEL of HCA-SX was determined by the authors at 1,240 mg/kg per day.

Mode of action

180. The cause of hepatotoxicity from *G. cambogia* is unclear. *In vitro* studies suggest that HCA may be toxic to the liver in high doses, but the rare instances of acute liver injury that occur with *G. cambogia* suggest an idiosyncratic form of injury. The possibility of mislabelling or adulteration with hepatotoxic herbal products has been identified as an issue in herbal related injury.

181. In the literature review by Crescioli et al., (2018), it was noted that HCA mechanism of toxicity is not clearly defined; however, HCA increases hepatic collagen accumulation, lipid peroxidation, mRNA levels of genes related to oxidative stress (superoxide dismutase and glutathione peroxidase), and inflammatory responses (tumour necrosis factor- α and monocyte chemoattractant protein-1). It was suggested that certain patients could have genetic predisposition leading to hepatotoxicity, such as cytochrome P450 polymorphisms promoting toxic accumulation of metabolites. The information suggested that there is a potential causal relationship between G. cambogia product exposure and development of herb induced liver injury (HILI); however, these were limited by the lack of data on factors influencing the severity of HILI, especially for cases derived from the literature.