COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Pyrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity

This Annex provides a summary of the carcinogenicity and mutagenicity data for individual Pas for which there is evidence for *in vivo* tumour formation.

Secretariat
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CLIVORINE

Carcinogenicity

***In vivo* study not performed to contemporary standards**

Kuhara et al. (1980) Cancer Letters 10: 117-122.

Method: A group of 6 male and 6 female ACI rats were given a 0.005% solution of clivorine as drinking water for 340 days and then continued on water until day 480 unless terminated as moribund. A group of 10 males and 10 female rats served as controls.

Major Findings: All treated animals survived to day 440 and 3 were still alive at termination. 2/12 rats had hemangioendothelial sarcoma in the liver and 6/12 had neoplastic nodules in the liver. 3/12 had interstitial cell tumors of the testis.
3 control animals died within 12 months of starting the experiment but none had tumours. Of the 17 remaining animals, none had liver tumours, 1/17 had an adenoma of the pituitary, 3/17 had interstitial cell tumours of the testis of which 1 also had a cortical adenoma of the adrenal, 1/17 had acinar cell adenoma of the pancreas.

Conclusion: Limited evidence for carcinogenicity.

Additional comments:

Mutagenicity

In vitro Bacterial

Yamanaka et al. (1979) Mutation Research 68:211-216.

Method: *S. typhimurium* TA100, TA92, TA1535, hisG46, TA1537 and TA98 (his frameshift) with or without PCB-induced rat or hamster S9 fraction was dosed with 0.2 – 2.0 mg/plate. The mixture of test compound, S9 and bacteria were pre-incubated prior to plating. A positive response was defined as induction of more than 100 revertants in excess of the spontaneous number.

Major Findings: Dose dependent increase in induction of mutation with TA100 and hamster or rat liver S9.
Negative with other strains – data not provided.

Conclusion: Positive

Additional comments:

In vitro Mammalian DNA damage – UDS

Mori et al. (1985) Cancer Research 45: 3125-3129.

Method: Hepatocytes were cultured following isolation either from ACI rats, C3H/HeN mice or Syrian Golden Hamsters. Hepatocytes were then attached to coverslips and exposed to clivorine for 20 hours. 50 cells per coverslip and 3 coverslips per treatment were assessed for DNA repair using net grain counts by autoradiography. Positive response required a net nuclear grain count of 5 above background and statistically greater than control.

Major Findings: Net nuclear grain count: Rat: Control: -0.3 ± 1.4, at 2 x 10^{-6} M: 8.6 ± 5.0, at 2 x 10^{-5} M: 20.5 ± 8.4 and at 2 x 10^{-4} M: Toxic.
Mouse: Control: -0.2 ± 1.3, at 2 x 10^{-6} M: -0.1 ± 1.1, at 2 x 10^{-5} M: 1.0 ± 1.4, and at 2 x 10^{-4} M: Toxic.
Hamster: Control: -0.2 ± 1.4, at 2 x 10^{-6} M: 14.7 ± 6.3, at 2 x 10^{-5} M: 5.2 ± 3.8 and at 2 x 10^{-4} M: Toxic

Conclusion: Positive in Rat and Hamster, Negative in Mouse

Additional comments: Cytotoxicity was also assessed.

DEHYDROHELIOTRIDINE

Carcinogenicity

Relevant *in vivo* studies

Peterson et al. (1983) Journal of the National Cancer Institute 70: 381-386.

Method: Groups of 24 male hooded rats were given dehydroheliotridine once every 4 weeks by intraperitoneal injection. First dose was 76.5 mg/kg, second dose was 65 mg/kg and remaining doses 60mg/kg. A control group received saline only. 9 injections were given over 32 weeks. Two rats from each group were killed at 10, 21 and 31 weeks after the first injection.

Major Findings: Strong suppression of growth after week 8. 18 rats died, 17 between weeks 61 and 96. 2 rats showed extensive neoplasia, 2 rats showed hemorrhage from polyarteritis lesions and 3 rats showed peritonitis, adhesions and fistulas associated with polyarteritis-induced necrotic intestinal lesions. In rats over 71 weeks 25% had parenchymal cell nuclei of 10-14μm in diameter (4% control) and small foci of regeneration were present. Of the 13 rats dying between weeks 61 and 80, 8/13 had moderate chronic progressive glomerulonephrosis, 8/13 had polyarteritis and 10 had interstitial cell tumours (11/12 in control).

Conclusion: Authors report that treated group had significantly higher tumour incidence than control, however, overall there was no convincing evidence of carcinogenicity.

Additional comments: Study also looked at thioacetamide and the joint effect of dehydroheliotridine and thioacetamide.

DEHYDRORETRONECINE

Carcinogenicity

***In vivo* study not performed to contemporary standards**

Mattocks & Cabral (1982) Cancer Letters 17: 61-66.

Method: 21 female LACA mice were given 5.0 µmol/mouse dehydroretronecine on their shaved backs at weekly intervals. A maximum of 47 doses were given. Surviving mice were killed at 102 weeks after the start of the experiment.

Major Findings: 20 treated animals were autopsied of which 8 had tumours. 5 skin tumours, 3 lung adenomas and 1 other tumour were found. 6 out of 20 control animals had tumours, 3 with lymphoma, 1 with lung adenoma and 2 with other tumours.

Conclusion: Significant increase in skin tumours compared to control.

Additional comments: Unpublished results in BALB/c mice are also described in this paper.

***In vivo* study not performed to contemporary standards**

Allen et al. (1975) Cancer Research 35: 997-1002.

Method: 75 male Sprague Dawley rats were given 20 mg/kg bw biweekly for 4 months and then 10 mg/kg bw biweekly for the next 8 months by subcutaneous injection. A control group of 50 animals was used. Treatment ceased at 12 months. Animals were kept until moribund in the 10 months after treatment finished.

After 4 months of treatment, 15 treated and 5 control rats were given a partial hepatectomy. After 36 hours regeneration, portions of liver were used to count mitotic indices

Major Findings: A decrease in body weight gain at 4 months lead the dose to be halved for the remainder of the study.

Mitotic indices per 1000 hepatocytes after partial hepatectomy: Control 61.7 ± 8.6. Treated 11.99 ± 6.6.

2 treated animals developed gross tumours (rhabdomyosarcoma) by the 11^{th} month of treatment. A further 29 rats developed rhabdomyosarcomas in the 10 months after the end of treatment. 5 rats with rhabdomyosarcomas had metastases.

Control data is not reported

Conclusion: The authors reported that dehydroretronecine caused tumours in this study.

Additional comments: This study provides evidence that treatment suppressed mitosis.

***In vivo* study not performed to contemporary standards**

Shumaker et al. (1976) Journal of the National Cancer Institute 56: 787-790.

Method: 60 male Sprague Dawley rats were given 20 mg/kg bw subcutaneously biweekly for 4 months and then 10 mg/kg bw subcutaneously biweekly for the next 8 months. A control group of 45 animals was used. Animals were sacrificed 12 months after cessation of treatment unless moribund.

Major Findings: A decrease in body weight gain at 4 months lead the dose to be halved for the remainder of the study.
2 treated rats developed circumscribed firm subcutaneous nodules at the injection site during the 11th month of treatment. An additional 37 animals developed tumours at the injection site (rhabdomyosarcomas) in the 12 months after treatment ceased. 5 of these 39 animals had metastases in the lung or heart.

Conclusion: The authors concluded that dehydroretronecine is carcinogenic in rats.

Additional comments:

***In vivo* study not performed to contemporary standards**
Johnson et al. (1978) Journal of the National Cancer Institute 61: 85-89.
Method: Female STS mice were divided into 4 groups. 15 animals formed the control group, 25 were given 20 mg/kg bw dehydroretronecine by topical application in 0.2 ml acetone (Group 1), 25 were given 20 mg/kg bw by subcutaneous injection (Group 2) and 75 mice received 20 mg/kg bw by topical application in 0.2 ml acetone and 20 mg/kg bw by subcutaneous injection (Group 3). Animals were treated once per week for 4 weeks and animals without tumours after 6 months were given an additional 2 weeks of dosing. Animals were sacrificed 15 months after the initiation of the experiment except where animals were moribund or developed palpable tumours before this.

Major Findings: Control group: 11 animals were still surviving at the time of first appearance of a skin tumour in a treated group (week 18). No skin tumours were found. 1 animal had a pulmonary adenoma.
Group 1: 16 mice were still surviving at week 39 when the first skin tumour appeared. 5 mice had basal cell carcinomas and 1 had squamous cell carcinoma. Other tumours found were fibrosarcoma in 2 animals, 1 with lymphoma, reticulum cell sarcoma and 1 had renal adenoma.
Group 2: 21 mice were still surviving at week 27 when the first skin tumour appeared. 11 had basal cell carcinoma of which 3 had metastases, 2 had squamous cell carcinoma of which 1 had metastasis. In addition, 1 animal had pulmonary adenocarcinoma.
Group 3: 55 mice were still surviving at week 18 when the first tumour appeared. 23 mice had basal cell carcinoma, of which 7 had metastases, 2 had squamous cell carcinoma, 1 had basosquamous cell carcinoma with lung metastases, 1 had a papilloma and 1 had a sebaceous adenoma. In addition, 1 had rhabdomyosarcoma, 2 had pulmonary adenocarcinoma, 1 had capillary hemangioma, 1 had hemangiosarcoma, 1 had lymphoma, 2 had myxoma and 1 had plasmocytoma.

Conclusion: The authors concluded that dehydroretronecine is capable of causing a large number of malignant skin tumours when administered topically or by subcutaneous injection.

Additional comments:

Mutagenicity

In vitro Bacterial

Ord et al. (1985) Mutation Research 149: 485-493.

Method: *Salmonella typhimurium* strain TA92 was tested at doses from 0 to 5000 µg/plate. 3 replicate experiments were carried out.

Major Findings: 0 µg/plate caused 42 ± 8 revertants per plate, 5 µg/plate caused 2 (only 1 replicate), 10 µg/plate caused 28 ± 10, 50 µg/plate caused 38 ± 6, 100 µg/plate caused 37 ± 9, 500 µg/plate caused 106 ± 33, 750 µg/plate caused 136 ± 50, 1000 µg/plate caused 159 ± 51, 2000 µg/plate caused 213 ± 36, 5000 µg/plate caused 210 ± 58 revertants per plate.

Conclusion: Positive

Additional comments: Mitomycin C 0 µg/plate caused 41 ± 8 revertants per plate, 0.01 µg/plate caused 46 ± 6, 0.05 µg/plate caused 52 ± 2, 0.1 µg/plate caused 76 ± 10, 0.5 µg/plate caused 78 ± 8, 1.0 µg/plate caused 107 ± 14, 2.5 µg/plate caused 97 ± 41, 5.0 µg/plate caused 90 ± 62, 10 µg/plate caused 139 ± 64, 15 µg/plate caused 29 and 25 µg/plate caused 27 ± 14 revertants per plate. At 15 µg/plate and over, most plates had no lawn colonies. Decarbamoylated mitomycin C 0 µg/plate caused 38 ± 11 revertants per plate, 1.0 µg/plate caused 32 ± 8, 2.5 µg/plate caused 91 ± 8, 5.0 µg/plate caused 153 ± 58, 10 µg/plate caused 211 ± 25, 15 µg/plate caused 273 ± 56, 25 µg/plate caused 425 ± 72, 35 µg/plate caused 495 ± 15, 50 µg/plate caused 484 ± 28, 100 µg/plate caused 266 ± 127 revertants per plate. At 250 µg/plate and over, most plates had no lawn colonies.

In vitro Mammalian cell Chromosome Aberration

Ord et al. (1985) Mutation Research 149: 485-493.

Method: Human peripheral blood was obtained from healthy donors. Lymphocytes were grown in tubes and placed in a non-gassed 37˚C incubator in a light-tight box for 72 hours. 5-Bromo-2'-deoxyuridine was added at 3 hours. Dehydroretronecine was added at 48 hours at 6 doses. Colchicine was added 1.5 hours before harvest and slides were prepared. 40 second generation mitotic spreads were scored for each dose from 2 separate experimental runs. SCE values refer to exchanges in the complete genome. Mitotic yield was calculated as mitotic figures divided by the number of stained nuclei with counts taken from a minimum of 1000 cells but continued until at least 20 mitotic figures were recorded.

Major Findings: 0 µM caused 8 ± 2 SCEs per mitotic spread, 0.75 µM caused 17.5 ± 5, 1.0 µM caused 19 ± 6, 2.5 µM caused 28 ± 6, 5.0 µM caused 47 ± 9, 7.5 µM caused 57 ± 10, 10 µM caused 66 ± 16 and 25 µM caused 78 ± 11

SCEs per mitotic spread. 50 µM contained no second generation mitoses. Mitotic yield was 0.6% at 0.75 and 1.0 µM, 1.0% at 2.5 µM, 1.2% at 5.0 µM, 0.9% at 7.5 µM, 0.5% at 10 µM and 0.4% at 25 µM.

Conclusion: Positive

Additional comments: Mitomycin C at 0.5 µM caused 40 ± 10 SCEs per mitotic spread.
The COM does not attach significant weight of evidence to sister chromatid exchange alone.

In vitro Mammalian DNA damage
Kim et al. (1995) Carcinogenesis 16: 2691-2697.
Method: Madin Darby bovine kidney epithelial cells CCL22 were labelled with ^{3}H thymidine for 22 hours and then treated with 300 µM and 500 µM dehydroretronecine for 2 hours. The cells were then exposed to 1000 rad of γ-irradiation before alkaline elution was performed. Cross-linking indices were derived comparing control cells with the treated cells.
The proportion of protein associated DNA cross links was assessed using alkaline elution with proteinase K.

Major Findings: Cross linking index at 300 µM 0.18 ± 0.01 and at 500 µM 0.18 ± 0.02. The proportion of protein associated DNA cross-links at 300 µM was 0.52 ± 0.26.

Conclusion: The authors concluded that dehydroretronecine was not a potent DNA cross-linker.

Additional comments: In addition, the DNA cross-linked with proteins in cells and nuclei was characterised in this paper.

LASIOCARPINE

Carcinogenicity

2 year – rat

NTP technical report 39

Method: Groups of 24 male and female F344 rats were given 0, 7, 15, 30 ppm in diet for 104 weeks.

Major Findings: Males: 30 ppm: all were dead by week 88 and all but 1 had tumours. 15 ppm: Decreased body weight in year 2 and all had tumours:.. 7 ppm: All but 1 had tumours
Females: 30 ppm: all were dead by week 69. 15 ppm: Decreased body weight in year 2 and 22/24 had tumours. 7 ppm: 23/24 had tumours.

Liver angiosarcoma: Male 30 ppm: 13/23 (57%) (also 1 in lung), 15 ppm: 11/23 (48%), 7 ppm: 5/24 (21%), 0 ppm: 0/23. Metastases in the lung were observed at all 3 doses (7, 5 and 3 respectively), in thymus at 15 ppm in 1 animal, in kidney in 1 animal at 7 ppm and in lymph node in 1 animal at 7 ppm and 15 ppm.
Female 30 ppm: 2/23 (9%) (quoted as 2/9 in main report), 15 ppm: 7/24 (29%) (also 2 in lung), 7 ppm: 8/22 (36%), 0 ppm: 0/24. Metastases in lung were observed in all 3 doses (1, 4 and 3 respectively), in adipose tissue in 1 animal at 30 ppm and in thymus in 1 animal at 7ppm.

Leukaemia and lymphoma: Male 30 ppm: 7/24, 15 ppm: 11/24, 7 ppm: 3/24 and 0 ppm: 3/24. (Not considered statistically significant)
Female 30ppm: 1/23, 15 ppm: 11/23, 7 ppm: 9/24, 0 ppm: 2/24.

Liver adenoma: Male 30 ppm: 3/23 (13%) also 2 with hepatocellular carcinoma, 15 ppm: 3/23 (13%), 7 ppm: 0/24, 0 ppm: 0/24.
Female 30 ppm: 6/23 (26%) also 1 with hepatocellular carcinoma, 15 ppm: 1/24 (4%), 7 ppm: 5/22 (23%), 0 ppm: 0/24.

There was a negative trend of interstitial-cell tumour of testis in males and a negative trend of chromophobe adenoma of pituitary in females.

In both sexes all treated groups, showed nodular hepatocyte hyperplasia.

Conclusion: The authors concluded that lasiocarpine was carcinogenic in F344 rats in this study.

Additional comments: In the 8 week range finding study, weight gain in males was unaffected at 10 and 20 ppm, 80% of that of controls at 40 ppm and 23 % of that of controls at 80 ppm. In females, weight gain was unaffected at 10 and 20 ppm, 72% of that of controls at 40 ppm and 14% of that of controls at [illegible]ppm.

***In vivo* study not performed to contemporary standards**

Rao & Reddy (1978) British Journal of Cancer 37: 289-293.

Method: Groups of 20 male F344 rats received a dietary concentration of 50 ppm for 55 weeks. Animals were killed at the end of week 59 unless dead before then. 10 control rats were killed at the end of week 59.

Major Findings: 17/20 Malignant tumours. 9/20 liver angiosarcoma (4 with lung metastases), 7/20 hepatocellular carcinoma (1 with lung metastasis), 1/20 adnexas skin tumour, 1/20 lymphoma. No tumours were observed in control animals.

Conclusion: Authors concluded that lasiocarpine induced liver angiosarcoma and hepatocellular carcinoma.

Additional comments: Study successfully transplanting tumours into weanling rats was carried out.

***In vivo* study not performed to contemporary standards**
Svoboda & Reddy (1972) Cancer Research 32: 908-912.
Method: Groups of 25 male F344 rats were given 0 or 7.8 (0.1 LD50) mg/kg bw by intraperitoneal injection twice weekly for 4 weeks and then once a week for 52 weeks. Animals were sacrificed when moribund (approx week 60-76 for treated animals).

Major Findings: Of the treated animals, 3 rats died of acute liver necrosis in 1st 4 weeks. 7 rats in total died in first 9 months. 16/18 developed tumours between week 60 & week 76. 10/16 had multiple tumours. 11/18 liver tumours (10 hepatocellular carcinoma, 1 cholangiocarcinoma). 6/18 squamous cell carcinoma of skin. 5/18 pulmonary adenoma. 2/18 adenocarcinoma of small intestine. 1/18 adenomyoma of ileum. 1/18 interstitial cell tumour of testis. All control animals survived. 2 lung adenomas were observed in this group.

Conclusion: Authors concluded that lasiocarpine is a carcinogen.

Additional comments: Study successfully transplanting tumours into weanling rats was carried out

***In vivo* study not performed to contemporary standards**
Svoboda & Reddy (1974) Journal of the National Cancer Institute 53: 1415-1418.
Method: 50 male Fischer rats were given 7.8 (0.1 LD50) mg/kg bw lasiocarpine by intraperitoneal injection twice weekly for 4 weeks and then once per week for 52 weeks. Surviving animals were sacrificed at 60 to 76 weeks.

Major Findings: 18 rats surviving at 56 weeks. 6/18 developed squamous cell carcinomas on the skin of the back.

Conclusion: Authors concluded that a malignant tumour formed following lasiocarpine administration.

Additional comments: Study successfully transplanting tumours into weanling rats was the main part of this paper.

Other relevant *in vivo* studies
Hayes et al. (1985) Cancer Research 45: 3726-3734.
Method: Male F344 rats were given up to 80 µmol/kg bw as a single or 2 doses by intraperitoneal injection.

Major Findings: Delayed and impaired regeneration after partial hepatectomy.

Conclusion: Poor initiator of γ-GT-positive resistant nodules.

Additional comments: Also other studies with partial hepatectomy and/or other carcinogen pretreatments with lasiocarpine.

***In vivo* study not performed to contemporary standards**
Schoental & Magee (1959) Acta Unio Int Contra Cancrum 15: 212-215.
Method: Single oral dose to rats; biopsies taken on day 3, 6, and 9

Major Findings: No liver necrosis at day 3, 6, or 9 but still develops typical liver lesion after 1 month (big parenchymal cells with enormous nuclei, early regeneration nodules with small infiltrating cells with darkly staining nuclei). Survival of between 26 days and 2.5 years. Historical controls and those for the experiment show such liver lesions

Conclusion:

Additional comments:

***In vivo* study not performed to contemporary standards**
Reddy & Svoboda (1972) Arch Path 93: 55-60.
Method: 20 F344 male rats were given 7.8 mg/kg bw by intraperitoneal administration twice weekly for 4 weeks and then once a week for 52 weeks. Laparotomies were performed at 4, 6, 10, 14 and 18 weeks and liver biopsies were taken.

Major Findings: Livers were smaller in size. At 6-9 weeks cells were enlarged. By 18 weeks there was mild portal fibrosis and proliferation of oveal cells. No cirrhosis or gross nodularity was observed.

Conclusion: Authors concluded that at 56 weeks, lasiocarpine was not found to be carcinogenic.

Additional comments: Studies also done with lasiocarpine combined with aflatoxin B1.

Mutagenicity
In vitro Bacterial
Yamanaka et al. (1979) Mutation Research 68: 211-216.

Method: *S. typhimurium* TA100, TA92, TA1535, hisG46, TA1537 and TA98 (his frameshift) with or without PCB-induced rat or hamster S9 fraction was dosed with 0.05 – 0.5 mg/plate. The mixture of test compound, S9 and bacteria were pre-incubated prior to plating. A positive response was defined as induction of more than 100 revertants in excess of the spontaneous number.

Major Findings: Dose dependent increase in induction of mutation with hamster liver S9 in strain TA100. With rat liver S9 in TA100, 0.05, 0.15 and 0.3 mg/plate induced 138, 133 and 153 revertants more than spontaneous numbers. At 0.5 mg/plate lasiocarpine was cytotoxic.
Negative with other strains – data not provided.

Conclusion: Positive in TA100 with S9

Additional comments:

In vitro Mammalian cell Gene mutation
Takanashi et al. (1980) Mutation Research 78: 67-77.
Method: Chinese hamster V79 cells were exposed to lasiocarpine for 48 hours (0, 5 x 10^{-4} or 1 x 10^{-3} M) or with S9 from sodium Phenobarbital-induced ACI or Sprague Dawley rats for 1 hour (0, 5 x 10^{-5} or 1 x 10^{-4} M). 10^5 cells were cultured for 2 days with control medium and then 10 days with 8 azo-guanine medium before colony counting. 5 dishes were assessed for each concentration.

Major Findings: Mutant frequency of 8 azoguanine resistant cells per 10^5 survivors: Without S9: Control: 2.1, at 5 x 10^{-4} M: 4.3 and at 1 x 10^{-3} M: 21.0. With S9: Control: 2.4, at 5 x 10^{-5} M: 9.2 and at 1 x 10^{-4} M: 20.8.

Conclusion: Positive with and without S9

Additional comments: Cytomorphical assessment also carried out.

In vitro Mammalian cell Chromosome Aberration
Takanashi et al. (1980) Mutation Research 78: 67-77.
Method: Chinese hamster V79 cells were exposed to 0, 2 x 10^{-4} or 5 x 10^{-4} M for 24 to 48 hours before 2 hours treatment with colcemid. 200 metaphases were analysed for chromosomal aberrations.

Major Findings: Aberrant metaphases: Control: 2.5% at 24 and 48 hours of which all were gaps. 2 x 10^{-4} M caused 5% at 24 hours of which all were gaps and 6% at 48 hours of which 5% was gaps and 1% was breaks. 5 x 10^{-4} M caused 5% at 24 hours of which 4.5% were gaps and 0.5% were breaks and 15% at 48 hours of which 14% were gaps and 1% was fragmentation.

Conclusion: Induced predominantly gaps.

Additional comments: Cytomorphical assessment also carried out.

In vivo Somatic cell Other
Furmanowa et al. (1983) Journal of Applied Toxicology 3: 127-130.
Method: Lateral roots of *Vicia faba* L. var *minor* were incubated for 21 hours in lasiocarpine solution of 3.2 x 10^{-3} M, 1.6 x 10^{-3} M and 0.8 x 10^{-3} M. Roots were assessed for chromosomal aberrations.

Major Findings: Metaphases with chromosomal aberrations: Control 1.0%, 0.8 x 10^{-3} M 4.9%, 1.6 x 10^{-3} M 5.8%, 3.2 x 10^{-3} M 7.5%.

Conclusion: Positive

Additional comments: The COM have not traditionally allocated a significant weight of evidence to these types of study.

In vivo Somatic cell Other
Culvenor et al. (1969) Annals New York Academy of Sciences 163:837-847.
Method: 5 mg lasiocarpine with 1.5 µc ^{14}C was administered by intraperitoneal injection to 1 rat and the distribution in the tissues was determined after 4 hours. Labelling of nucleic acids was assessed.

Major Findings: Labelling of DNA is equivalent to one aminoalcohol unit / 160,000 nucleotides.

Conclusion: Limited evidence of DNA binding

Additional comments:

MONOCROTALINE

Carcinogenicity

In vivo study not to contemporary standards

Shumaker et al. (1976) Journal of the National Cancer Institute 56: 787-790.

Method: 60 male Sprague Dawley rats were given 5 mg/kg bw subcutaneously biweekly for 12 months. A control group of 45 animals was used. Animals were sacrificed 12 months after cessation of treatment unless moribund.

Major Findings: Tissue changes in treated rats: 10/60 had pulmonary adenocarcinoma, 1/60 pulmonary adenocarcinoma with metastases, 5/60 hepatocellular carcinoma, 3/60 acute myelogenous leukaemia, 4/60 rhabdomyosarcoma, 8/60 adrenal adenoma, 1/60 renal adenoma, 8/60 peliosis hepatis, 3/60 bile duct hyperplasia.
In control rats 2/45 had adrenal adenoma but otherwise no tumours were reported.

Conclusion: The authors conclude from this study that monocrotaline is carcinogenic in rats.

Additional comments: Historical control rate of hepatocellular carcinoma in male Sprague Dawley rats is in the range 0-3.4% (Baldrick 2005 Toxicologic Pathology 33: 283-291)

In vivo study not to contemporary standards

Allen et al. (1975) Cancer Research 35: 997-1002.

Method: 75 male Sprague Dawley rats were given 5 mg/kg bw biweekly for 12 months by subcutaneous injection. A control group of 50 animals was used. Treatment ceased at 12 months. Animals were kept until moribund in the 10 months after treatment finished.
After 4 months of treatment, 15 treated and 5 control rats were given a partial hepatectomy. After 36 hours regeneration, portions of liver were used to count mitotic indices

Major Findings: Mitotic indices per 1000 hepatocytes after partial hepatectomy: Control 61.7 ± 8.6. Treated 9.47 ± 2.5.
2 treated animals developed gross tumours (rhabdomyosarcoma) 6 and 8 months after cessation of treatment. Another 4 animals dying in the 9^{th} and 10^{th} month after treatment showed neoplasms 2/60 had hepatocellular carcinoma, 2/60 acute myelogenous leukaemia and 2/60 pulmonary adenoma.
Control data is not reported

Conclusion: The authors reported that monocrotaline caused tumours in this study.

Additional comments: This study provides evidence that treatment suppressed mitosis.

Mutagenicity

In vitro Bacterial

Yamanaka et al. (1979) Mutation Research 68: 211-216.

Method: *S. typhimurium* TA100, TA92, TA1535, hisG46, TA1537 and TA98 (his frameshift) with or without PCB-induced rat or hamster S9 fraction was dosed with 0.2 – 2.0 mg/plate. The mixture of test compound, S9 and bacteria were pre-incubated prior to plating. A positive response was defined as induction of more than 100 revertants in excess of the spontaneous number.

Major Findings: Negative – data not provided.

Conclusion: Negative

Additional comments:

In vitro Bacterial

White et al. (1984) Journal of Animal Science 58: 1245-1254.

Method: *S. typhimurium* TA1535, TA100, TA1537 and TA98 with or without Aroclor 1254-induced male rats dosed with 0, 5, 50 or 500 µg/plate. A positive response was defined as twice the revertant number of the control and a toxic response was defined as half the revertant number of the control. All positive and toxic responses were replicated for verification.

Major Findings: Negative – the ratio of revertants was between 0.7 and 1.8 for PA test solutions used one of which was monocrotaline.

Conclusion: Negative

Additional comments:

In vitro Bacterial

Green & Muriel (1975) Mutation Research 28: 331-336.

Method: *Eschericia coli* strains WP2, WP2 *uvrA*, WP67, CM 561, CM571, CM611 and WP100 were dosed with 10 mg/ml with sodium phenobarbitone-induced male rat liver microsomal fraction. Survival was compared to control and Trp+ revertants in WP2 and WP2*uvrA* strains were measured.

Major Findings: Survival range: WP2: 32 – 110% (n=3), WP2 *uvrA*: 64-112% (n=4), WP67: 68-100% (n=3), CM561: 43-93% (n=3), CM571: 52-75% (n=3), CM611: 2.0-31% (n=4) and WP100 0.5-13% (n=4).
CM611 and WP100 strains are most sensitive and WP2 *uvrA*, CM561 and CM571 strains are slightly sensitive.
Number of Trp+ revertants showed an absolute increase with monocrotaline (but not above 1.5 fold which would be considered significant).

Conclusion: Negative

Additional comments: Authors suggest that experimental conditions were not optimal for activation of monocrotaline.

In vitro Mammalian cell Chromosome Aberration
Müller et al. (1992) Mutation Research 282: 169-176.
Method: V79 cells alone were treated continuously for 18hours when the cells were harvested. Additionally, V79 cells were treated with S9 mix from Aroclor-induced rats for 2 hours before being washed twice and then reincubated for 16 hours. Primary hepatocytes isolated from Wistar rats by two step perfusion were cocultured with V79 cells and these cultures were treated for 18 hours. Concentrations of monocrotaline ranged from 3.16×10^{-6} to 10^{-3} M.
Cultures were incubated with colcemid for 2 hours, mitotic cells were shaken off and the slides prepared. 100 metaphases were assessed for chromosomal aberrations where possible. Gaps and isogaps were scored but not included in the calculation of damaged cells. P-values below 0.01 indicated a significant difference.
Cytotoxicity is expressed as reduced number and/or poor quality metaphases.

Major Findings: Aberrant metaphases: Without exogenous metabolism: DMSO control showed 1.8% aberrant metaphases not including gaps. Treated cells showed increasing response from 0.5% at 3.16×10^{-6} M to 3.0% at 10^{-3} M none of which were significant. No cytotoxic effects were observed.
With rat liver S9 mix: DMSO control showed 1.8%. Treated cells showed 1.8% at 3.16×10^{-6} M, 0.3% at 10^{-5} M, 1.3% at 3.16×10^{-5} M, 2.8% at 10^{-4} M and 4.8% at 3.16×10^{-4} M and 21.5% at 10^{-3} M. Responses at 3.16×10^{-4} M and 10^{-3} M were considered significant. No cytotoxic effects were observed.
With rat hepatocytes: DMSO control showed 2.0%. Treated cells showed 0.5% at 3.16×10^{-6} M, 2.8% at 10^{-5} M, 14.3% at 3.16×10^{-5} M, 26.5% at 10^{-4} M and 45.7% at 3.16×10^{-4} M and 56.4% at 10^{-3} M. Responses at 3.16×10^{-5} M and higher were considered significant. No cytotoxic effects were observed except at high concentration which showed low numbers of scorable metaphases.

Conclusion: Positive

Additional comments: Mitomycin C showed 33.0% aberrant metaphases without external metabolism. Cyclophosphamide showed 30.0% aberrant metaphases with rat liver S9 mix and 7.0% with rat hepatocytes. The low response of cyclophosphamide with rat hepatocytes was suggested to be due to a low effect in one experiment of the duplicates tested.

In vitro Mammalian cell Chromosome Aberration
Bruggeman & van der Hoeven (1985) Mutation Research 142: 209-212
Method: V79 cells were co-cultured with chick embryos and exposed to doses of 0, 1.2, 2.5, 3.5 or 5.0 µg/ml of monocrotaline. Cells were then treated with BrdUrd for 24 hours and then colchicine for 4hours before assessment. 25 metaphases per slide were scored for sister chromatid exchanges.

Major Findings: SCEs per chromosome: Control 0.27 ± 0.02, Treated cells: at 1.2 µg/ml showed 0.54 ± 0.04, at 2.5 µg/ml 0.74 ± 0.05, at 3.5 µg/ml 1.04 ± 0.10 and at 5.0 µg/ml 1.26 ± 0.10.

Conclusion: Authors concluded that monocrotalineis a strong inducer of sister chromatid exchange in C79 cells with chick embryo hepatocytes.

Additional comments: Positive control 3-methylcholanthrene at 1.0 µg/ml caused 0.55 ± 0.04 SCEs per chromosome. The COM does not attach weight of evidence to sister chromatid exchange alone.

In vitro Mammalian DNA damage – UDS
Mori et al. (1985) Cancer Research 45: 3125-3129.
Method: Hepatocytes were cultured following isolation either from ACI rats, C3H/HeN mice or Syrian Golden Hamsters. Hepatocytes were then attached to coverslips and exposed to senkirkine for 20 hours. 50 cells per coverslip and 3 coverslips per treatment were assessed for DNA repair using net grain counts by autoradiography. Positive response required a mean net nuclear grain count of 5 above background and statistically greater than control. Cytotoxicity was determined by detachment of cells, absence of S-phase cells and general morphology.

Major Findings: Net nuclear grain count: Rat: Control -0.3 ± 1.4. Treated cells at 2×10^{-6} M showed 4.3 ± 3.0, at 2×10^{-5} M 15.2 ± 4.9 and toxicity at 2×10^{-4} M. Mouse: Control -0.2 ± 1.3. Treated cells at 2×10^{-6} M showed 0.5 ± 1.0, at 2×10^{-5} M 1.8 ± 1.6 and at 2×10^{-4} M 5.8 ± 4.6. Hamster: Control -0.2 ± 1.4. Treated cells at 2×10^{-6} M showed 5.1 ± 3.7, at 2×10^{-5} M 5.5 ± 5.2 and at 2×10^{-4} M 9.0 ± 5.2.

Conclusion: Positive in Rat, Mouse and Hamster

Additional comments: Positive control 9,10-dimethyl-1,2-benz(a)anthracene at 10^{-6} M showed 6.7 ± 4.1 net nuclear grain count, at 10^{-5} M 24.2 ± 7.3 and was toxic at 10^{-4} M in rat hepatocytes.
In mice at 10^{-6} M showed 1.2 ± 1.6 net nuclear grain count, at 10^{-5} M 3.8 ± 2.8 and at 10^{-4} M 13.9 ± 4.9.
In hamsters at 10^{-6} M showed 7.1 ± 5.2 net nuclear grain count, at 10^{-5} M 32.1 ± 11.4 and at 10^{-4} M 67.0 ± 17.4.

In vitro Mammalian DNA damage – UDS
Berry et al. (1996) Journal of Natural Toxins 5: 7-24.
Method: Hepatocytes isolated from male Sprague Dawley rats by perfusion were seeded onto glass coverslips and allowed to attach for 2 hours. Cells were exposed to 1.0 µM, 5.0 µM and 10.0 µM monocrotaline with ^{3}H-thymidine for 18-20 hours. 150-200 nuclei per coverslip and 3 slides per treatment were assessed using an image analysis system. A net nuclear grain count more than 5 above background were considered positive and S-phase cells were ignored.

Major Findings: 1.0 µM gave a net nuclear grain count of 5, considered equivocal. 5.0 µM gave a count of 18 and 10.0 µM a count of 20 both of which were considered positive.

Conclusion: Positive.

Additional comments: Cell viability was determined using LDH release.
45 µM benzo(a)pyrene or 100 µM acetylaminofluorene were used as a positive control and gave net nuclear grain counts of 38 and greater than 50 respectively.

In vitro Mammalian DNA damage
Hincks et al. (1991) Tox Appl Pharm 111: 90-98
Method: Madin Darby bovine kidney epithelial cell line CCL 22 were labelled with 3H-thymidine for 22 hours. Medium was replaced with medium containing 50-500 µM PA. DNA cross-links were assessed and cross-link indices derived by comparing treated response to control.

Major Findings: DNA cross link indices: 50 µM – Not detected,100 µM – 0.068, 300 µM – 0.091, 500 µM – 0.201, Interstrand cross links at 300 µM – 0.049.

Conclusion: Positive dose-related response.

Additional comments: Cell viability determined by tryphan blue exclusion but no cytotoxicity was reported.
No positive control used.

In vivo Somatic cell Micronucleus
Sanderson & Clark (1993) Mutation Research 285: 27-33.
Method: 4 pregnant female Swiss-albino mice received a single intraperitoneal injection of 125 mg/kg bw (0.75 LD50) monocrotaline (5 control animals received physiological saline). Animals were sacrificed 21 hours after injection. 2 foetuses were taken from the right where animals had been injected on the left and 2 femora were removed. Bone marrow cells were collected from the femorea and liver cells from the foetuses. Slides were prepared and 1000 polychromatic erythrocytes were scored for the presence or absence of micronuclei. Any normochromatic erythrocytes in the same fields were also scored.

Major Findings: No deaths were reported and signs of toxicity were not reported.
Adult bone marrow cells: Polychromatic cells with micronuclei in control mice was 0.7 ± 0.3% and in treated mice 2.0 ± 0.3% (significantly different from control).
Normochromatic cells with micronuclei in control mice was 0.6 ± 0.2% and in treated mice 0.9 ± 0.5%.
Erythrocyte population which was polychromatic in control mice was 57.1 ± 2.4% and in treated mice 69.8 ± 2.7%.

Fetal liver cells: Polychromatic cells with micronuclei in control mice was 0.9 ± 0.3% and in treated mice 3.6 ± 0.3% (significantly different from control). Normochromatic cells with micronuclei in control mice was 1.0 ± 0.2% and in treated mice 1.2 ± 0.6%.
Erythrocyte population which was polychromatic in control mice was 58.8 ± 7.5% and in treated mice 85.1 ± 4.0% (significantly different from control).

Conclusion: Authors report a positive response, however the dose level used was very high.

Additional comments: It is difficult to assess the toxicity in this study and it is not possible to assess the mode of action.

In vivo Somatic cell DNA damage
Wang et al. (2005) Cancer Letters 226: 27-35.
Method: Female F344 rats were given a single dose of 10 mg/kg bw monocrotaline by oral gavage. Groups of 4 were sacrificed at 8 hours, 1, 2, 3, 7 and 30 days after dosing. ^{32}P-postlabelling/HPLC analysis was performed and quantification was based on 2 epimeric DHP-3'-dGMP standards.

Major Findings: DHP-derived DNA adducts formed from 1 day onwards, peaked at 7 days before dropping to 30 days. At all time points levels of adducts was approximately 8-fold lower than with riddelliine (positive control).

Conclusion: Positive

Additional comments: DHP and N-oxide formation from monocrotaline was investigated using uninduced rat liver microsomes, dexamethasone-induced rat liver microsomes and dexamethasone-induced rat lung microsomes.

In vivo Somatic cell DNA damage
Wang et al. (2005) Toxicology Letters 155: 411-420.
Method: 3 Female F344 rrats were dosed with 3.0 µmol/kg bw/day for 3 consecutive days. Animals were sacrificed 24 hours after final dosing and liver tissues stored. DHP-derived DNA adducts were analysed by ^{32}P-postlabelling/HPLC method using a DHP-3'-dGMP standard.

Major Findings: 55.2 ± 1 DHP-derived DNA adducts per 10^7 nucleotides were found.

Conclusion: Positive

Additional comments: Signs of toxicity were not reported.

PETASITENINE

Carcinogenicity

In vivo study not performed to contemporary standards

Hirono et al. (1977) Journal of the National Cancer Institute 58: 1155-1157.

Method: Group 1 of 3 male ACI rats received 0.05% solution in distilled water for 45 days. Group 2 of 5 male and 6 female ACI rats received 0.01% solution until termination. A control group of 10 male and 9 female ACI rats received tap water. The experiment was terminated at day 480.

Major Findings: Group 1 – The 3 rats were killed due to moribund condition after 72 days with the liver showing liver cell necrosis, hemorrhage and bile duct proliferation. Group 2 – 10/11 rats survived beyond day 160 (1 died of lung abscess at day 53). 5/10 developed liver hemangioendothelial sarcomas of which 1 had a metastasis. 5 animals had liver cell adenomas, 2 of which also had hemangioendothelial sarcomas. Control – 1/19 had subcutaneous fibrosarcoma.

Conclusion: Limited evidence for carcinogenicity

Additional comments:

Mutagenicity

In vitro Bacterial

Yamanaka et al. Mutation Research 68: 211-216.

Method: *S. typhimurium* TA100, TA92, TA1535, hisG46, TA1537 and TA98 (his frameshift) with or without PCB-induced rat or hamster S9 fraction was dosed with 0.2 – 2.0 mg/plate.

Major Findings: Positive with TA100 and S9. Negative with other strains.

Conclusion: Positive

Additional comments:

In vitro Mammalian cell Gene mutation

Takanashi et al. (1980) Mutation Research 78: 67-77.

Method: Chinese hamster V79 cells were exposed to petasitenine for 48 hours or with S9 from sodium Phenobarbital-induced ACI or Sprague Dawley rats for 1 hour. 10^5 cells were cultured for 2 days with control medium and then 10 days with 8 azo-guanine medium before colony counting. 5 dishes were assessed for each concentration.

Major Findings: Mutant frequency of 8 azoguanine resistant cells per 10^5 survivors: Without S9: Control: 2.1, at 5×10^{-3} M: 3.9 and at 1×10^{-2} M: 4.8. With S9: Control: 2.4, at 1×10^{-3} M: 15.7 and at 2×10^{-3} M: 70.6.

Conclusion: Positive with S9

Additional comments: Cytomorphical assessment also carried out.

In vitro Mammalian cell Chromosome Aberration
Takanashi et al. (1980) Mutation Research 78: 67-77.
Method: Chinese hamster V79 cells were exposed for 24 to 48 hours before 2 hours treatment with colcemid. 200 metaphases were analysed for chromosomal aberrations.

Major Findings: Aberrant metaphases: Control 2.5% at 24 and 48 hours all of which are gaps. 5 x 10^{-3} M caused 10.5% at 24 hours of which 9.5% are gaps, 1% are exchanges and 1.5% fragmentation and 28% at 48 hours of which 21% are gaps, 4.5% are breaks, 4.5% are exchanges and 3% are fragmentation. 1 x 10^{-2} M caused 23.5% at 24 hours of which 15.5% are gaps, 1.5% are breaks, 2.5% are exchanges and 5.5% are fragmentation and 26% at 48 hours of which 10.5% are gaps, 6% are breaks, 2% are exchanges and 11.5% are fragmentation.

Conclusion: Positive

Additional comments: Cytomorphical assessment also carried out.

In vitro Mammalian DNA damage – UDS
Mori et al. (1985) Cancer Research 45: 3125-3129.
Method: Hepatocytes were cultured following isolation from ACI rats. Hepatocytes were then attached to coverslips and exposed to petasitenine for 20 hours. 50 cells per coverslip and 3 coverslips per treatment were assessed for DNA repair using net grain counts by autoradiography. Positive response required a net nuclear grain count of 5 above background and statistically greater than control.

Major Findings: Net nuclear grain count: Control: -0.3 ± 1.4, at 2 x 10^{-6} M: 3.0 ± 2.9, at 2 x 10^{-5} M: 15.1 ± 6.1 and at 2 x 10^{-4} M Toxic.

Conclusion: Positive

Additional comments: Cytotoxicity was also assessed.

SENKIRKINE

Carcinogenicity

In vivo study not performed to contemporary standards

Hirono et al. (1979) Journal of the National Cancer Institute 63: 469-472.

Method: Groups of 20 male ACI rats were given intraperitoneal injections of senkirkine at 0 or 22 (0.1 LD50) mg/kg bw twice weekly for 4 weeks and then once per week for 52 weeks.

Major Findings: All treated animals survived to 290 days then deaths occurred up to termination at 650 days. 45% (9) showed liver cell adenoma. 2 showed other tumours (myeloid leukaemia and interstitial cell tumour of the testis). Controls showed no liver tumours but 3 had other tumours (1 myeloid leukaemia, 1 fibroma of the back, 1 interstitial cell tumour of the testis).

Conclusion: Authors considered that compared to a similar study on lasiocarpine, the resulting tumour incidence was low.

Additional comments:

Mutagenicity

In vitro Bacterial

Yamanaka et al. Mutation Research 68: 211-216.

Method: *S. typhimurium* TA100, TA92, TA1535, hisG46, TA1537 and TA98 (his frameshift) with or without PCB-induced rat or hamster S9 fraction was dosed with 0.2 – 2.0 mg/plate. The mixture of test compound, S9 and bacteria were pre-incubated prior to plating. A positive response was defined as induction of more than 100 revertants in excess of the spontaneous number.

Major Findings: Dose dependent increase in induction of mutation with TA100 and hamster or rat liver S9 except at 2 mg/plate with rat liver S9 where the number of revertants is decreased compared to the next highest dose. Negative with other strains – data not provided.

Conclusion: Positive in TA100 with S9

Additional comments:

In vitro Mammalian cell Gene mutation

Takanashi et al. (1980) Mutation Research 78: 67-77.

Method: Chinese hamster V79 cells were exposed to senkirkine for 48 hours or with S9 from sodium Phenobarbital-induced ACI or Sprague Dawley rats for 1 hour. 10^5 cells were cultured for 2 days with control medium and then 10 days with 8 azo-guanine medium before colony counting. 5 dishes were assessed for each concentration.

Major Findings: Mutant frequency of 8 azoguanine resistant cells per 10^5 survivors: Without S9: Control: 2.1, at 5×10^{-3} M: 3.4 and at 1×10^{-2} M: 4.5. With S9: Control: 2.4, at 2×10^{-4} M: 5.4 and at 5×10^{-4} M: 47.4.

Conclusion: Positive with S9

Additional comments: Cytomorphical assessment also carried out.

In vitro Mammalian cell Chromosome Aberration

Takanashi et al. (1980) Mutation Research 78: 67-77.

Method: Chinese hamster V79 cells were exposed for 24 to 48 hours before 2 hours treatment with colcemid. 200 metaphases were analysed for chromosomal aberrations.

Major Findings: Aberrant metaphases: Control 2.5% at 24 and 48 hours all of which were gaps. 5 x 10^{-3} M caused 5.5% at 24 all of which were gaps and 5.5% at 48 hours of which 5% were gaps and 0.5% were breaks. 1 x 10^{-2} M caused 2.5% at 24 hours and 6.5% at 48 hours all of which were gaps.

Conclusion: Negative

Additional comments: Cytomorphical assessment also carried out.

In vitro Mammalian cell Chromosome Aberration

Bruggeman & van der Hoeven (1985) Mutation Research 142: 209-212

Method: V79 cells were co-cultured with chick embryos and exposed to doses of 0, 0.6, 1.2, 2.5 or 5.0 µg/ml of senkirkine. Cells were then treated with BrdUrd for 24 hours and then colchicine for 4hours before assessment. 25 metaphases per slide were scored for sister chromatid exchanges.

Major Findings: SCEs per chromosome: Control 0.27 ± 0.02, 0.6 µg/ml 0.95 ± 0.08, 1.2 µg/ml 1.28 ± 0.09, 2.5 µg/ml 1.45 ± 0.10, 5.0 µg/ml 1.86 ± 0.16.

Conclusion: Positive

Additional comments: The COM does not attach significant weight of evidence to sister chromatid exchange alone.

In vitro Mammalian DNA damage – UDS

Mori et al. (1985) Cancer Research 45: 3125-3129.

Method: Hepatocytes were cultured following isolation either from ACI rats, C3H/HeN mice or Syrian Golden Hamsters. Hepatocytes were then attached to coverslips and exposed to senkirkine for 20 hours. 50 cells per coverslip and 3 coverslips per treatment were assessed for DNA repair using net grain counts by autoradiography. Positive response required a net nuclear grain count of 5 above background and statistically greater than control.

Major Findings: Net nuclear grain count: Rat: Control: -0.3 ± 1.4, at 2 x 10^{-6} M: 3.9 ± 4.1, at 2 x 10^{-5} M: 24.0 ± 11.6 and at 2 x 10^{-4} M Toxic.
Mouse: Control: -0.2 ± 1.3, at 2 x 10^{-6} M: 0.1 ± 1.1, at 2 x 10^{-5} M: 1.5 ± 1.9 and at 2 x 10^{-4} M: 7.6 ± 5.8.
Hamster: Control: -0.2 ± 1.4, at 2 x 10^{-6} M: 3.2 ± 2.8, at 2 x 10^{-5} M: 4.9 ± 3.4 and at 2 x 10^{-4} M: 6.7 ± 4.5.

Conclusion: Positive in Rat, Mouse and Hamster

Additional comments: Cytotoxicity was also assessed.

In vivo Somatic cell Other
Candrian et al. (1984) Food Chemical Toxicology
Method: Sex-linked recessive lethal assay was carried out using 150 male *Drosophila melanogaster* fed 10^{-5} M senkirkine.

Major Findings: % Lethals (Broods 1-3) In control: 0.17, at 10^{-5} M: 4.4 of which brood 1: 1.2, brood 2: 9.3 and brood 3: 4.9.
Number of lethals per number of chromosomes (Broods 1-3) In control: 15/9081, at 10^{-5} M: 112/2541 of which Brood 1: 15/1225, brood 2: 69/741 and brood 3: 28/575

Conclusion: Positive

Additional comments: The COM does not attach significant weight of evidence to these types of study.

SYMPHITINE

Carcinogenicity

In vivo study not to contemporary standards

Hirono et al. (1979) Journal of the National Cancer Institute 63: 469-472.

Method: Groups of 20 male ACI rats were given intraperitoneal injections of symphytine at 0 or 13 (0.1 LD50) mg/kg bw twice weekly for 4 weeks and then once per week for 52 weeks.

Major Findings: All treated animals survived to 330 days then deaths occurred up to termination at 650 days. 5% (1) showed liver cell adenoma. 15% (3) showed liver hemangioendothelial sarcoma and 1 showed myeloid leukaemia Controls showed no liver tumours but 3 had other tumours (1 myeloid leukaemia, 1 fibroma of the back, 1 interstitial cell tumour of the testis).

Conclusion: Authors considered that compared to a similar study on lasiocarpine, the resulting tumour incidence was low.

Additional comments