REACH monitor

Anthraquinone

Genotoxicity and carcinogenicity potential of different manufacturing origins

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1.- INTRODUCTION

Genotoxic and carcinogenic potential of Anthraquinone has been discussed. There are several studies available on anthraquinone. However their validity to assess potential effects is contradictory.

It is stated by different authors that these differences are due to contaminants related to anthraquinone manufacture methods. Therefore available data should be taken with care and distinguished by substance's origin.

This report shows all available data according to this perspective including metabolism and analogues widely studied.

2.- MANUFACTURING METHOD AND IMPURITIES

Commercial anthraquinone (AQ) is produced by at least three different production methods:

- Oxidation of anthracene (AQ-OX)
- Friedel-Crafts technology (AQ-FC)
- Diels-Alder chemistry (AQ-DA)

AQ-OX begins with anthracene produced from coal tar and different lots can contain various contaminants, particularly the mutagenic isomers of nitroanthracene that can be found at concentrations >2500 p.p.m. (Butterworth, 2001; US EPA, 1977; ICI, 1978a, 1978b).

AQ-FC is prepared from Friedel-craft reaction of benzene and phthalic anhydride in presence of $AlCl_3$. The resulting o-benzoylbenzoic acid then undergoes cyclization, forming anthraquinone.

The Diels-Alder reaction of naphthoquinone and butadiene followed by oxidative dehydrogenation will also produce 9,10-anthraquinone.

3.- METABOLISM

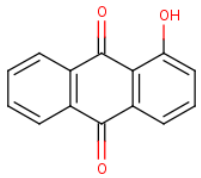
Main metabolites of anthraquinone are 1-hydroxyanthraquinone and 2-hydroxyanthraquinone (Sato *et al.*, 1956;).

In male F344 rats, [14C]anthraquinone was well absorbed (> 99.8% of the administered dose) from the gastrointestinal tract following oral doses ranging from 0.35 to 350 mg/kg bw. Following absorption, anthraquinone was distributed to various tissues, with highest concentrations in the adipose tissue. 2-Hydroxyanthraquinone was the major metabolite detected in the urine of rats exposed to anthraquinone intravenously or orally (NTP, 2005).

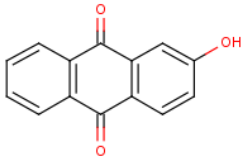
Single-dose toxicokinetic studies have been conducted in F344/N rats and B6C3F1 mice (NTP, 2005). Intravenous administration showed biphasic curves in both male and female rats that are best described as a two-compartment open model, and the half-life for plasma

anthraquinone was 10–12 hours. Oral administration by gavage also gave results that best fit a two-compartment model. Maximum concentration values were proportional to dose and were observed at 8–18 hours. The half-life could not be estimated reliably at higher doses. The areas under the curve were also proportional to dose. In mice, similar two-compartment behavior was estimated with a half-life in plasma of 4 hours following intravenous administration. Following oral administration to mice, the half life was 4–6 hours and the areas under the curve were proportional with dose.(IARC monophaps 101).

A metabolism study was conducted using male Fischer 344 rats in which they were fed formulations of 4 lots of anthraquinone, produced by three different synthetic routes: (AQ-OX, AQ-DA and AQ-FC). 1 and 2-hydroxyanthraquinone and anthraquinone were found in all samples from the dosed animals, with 2- hydroxyanthraquinone ten folds the concentration of 1- hydroxyanthraquinone. Both metabolites were found to be 65% less concentrated in AQ-FC than in AQ.OX (Graves et al 2005).



1- hydroxyanthraquinone



2-hydroxyanthraquinone

Metabolites and genotoxicity.

As per anthraquinone, there are some studies on genotoxicity for hydroxyanthraquinones with contradictory results. Most of these studies are not detailing the purity of the tested substance.

1- hydroxyanthraquinone:

An Ames test show negative results in Salmonella (Brown et al, 1976). A repeat dose toxicity study with 60 male rats dosed 1% 1-hydroxyanthraquinone during 480 days show tumors in liver, stomach and large intestine (Mori et al, 1990).

2- hydroxyanthraquinone:

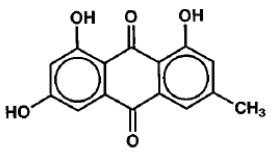
Ames test in Salmonella was positive in two strains with metabolic activation (Tikkanen, 1983). In another Ames test it was positive in TA100 with metabolic activation and negative with TA98 (Butterworth, 2004). Positive result was obtained in Salmonella TA98 with and without metabolic activation and negative in TA100 (NTP, 2005).

Purified 1-OH-AQ and 2-OH-AQ exhibited only weak mutagenic activity in selected strains of tester bacteria and required S9 (Butterworth, 2004).

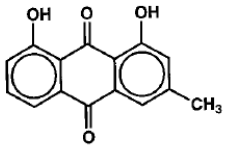
Can be concluded that 1 and 2- hydroxyanthraquinones needs metabolic activation to show slighlyt mutagenic potential.

4.- ANALOGUES

There are some structural analogues of anthraquinone that have been studied for their genotoxic potential as emodin (1,3,8-trihydroxy-6-methylanthraquinone) and chrysophanol (1,8-dihydroxy-3-methylanthraquinone).



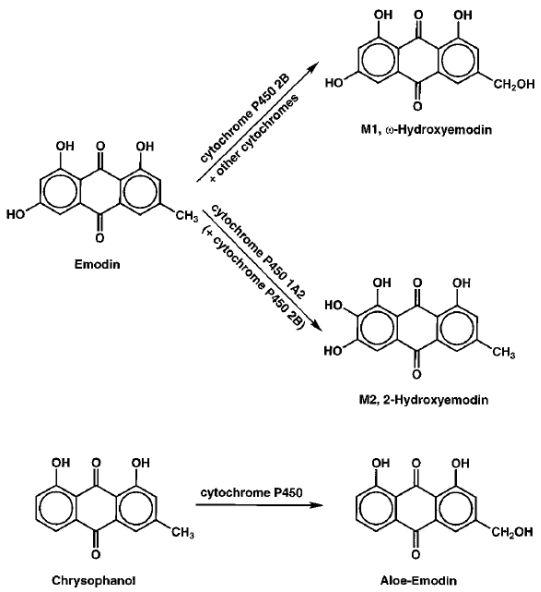
Emodin



Chrysophanol

Metabolites and genotoxicity.

Metabolites of emodin are v-hydroxyemodin and 2-hydroxyemodin (Mueller 1998; Murakami 1987; Masuda, 1984) and chrysophanol is transformed, in a cytochrome P450-dependent oxidation, to aloe-emodin (1,8-dihydroxy-3-hydroxymethylanthraquinone) as the major product formed (Stephan et al. 1998).



Metabolic pathway of emodin and chrysophanol (from Stephan et al. 1998)

2-hydroxyemodin (1,2,3,8-tetrahydroxy-6-methyl-anthraquinone), was proved to be a direct mutagen to Salmonella TA1537 (Masuda, 1984)

The mutagenicity of the parent dihydroxyanthraquinones and their metabolites was compared in the *in vitro* micronucleus test in mouse lymphoma L5178Y cells. 2-Hydroxyemodin induced much higher micronucleus frequencies, compared with emodin. v-Hydroxyemodin induced lower micronucleus frequencies, compared with emodin. Aloe-emodin induced significantly higher micronucleus frequencies than did chrysophanol. These data indicate that the cytochrome P450- dependent biotransformation of emodin and chrysophanol may represent bioactivation pathways for these compounds (Stephan et al. 1998).

Some emodin metabolites and some other 1,8-dihydroxyanthraquinones have genotoxic properties. Cytochrome P450-dependent biotransformation reactions for these compounds may thus represent bioactivation reactions but may also result in detoxication. Emodin itself is only a weak inducer of micronuclei. 2-Hydroxylation of emodin must be regarded as a bioactivation reaction, because the 2-hydroxyemodin formed is significantly more potent in micronucleus induction. Moreover, the biotransformation of chrysophanol by cytochromes P450 to yield aloe-emodin must also be regarded as a bioactivation reaction, because aloe-emodin is a much more potent inducer of micronuclei than is chrysophanol. In contrast, hydroxylation of the exocyclic methyl group in emodin may be regarded as a detoxication reaction, because the formed v-hydroxyemodin has a lower potential for the induction of micronuclei.

5.- AVAILABLE STUDIES

AQ has been reported to be negative in a variety of genotoxicity tests including numerous Ames mutagenicity assays. In addition, it is reported that AQ-DA is negative in the *Salmonella-Escherichia coli* reverse mutation assays, the L5178Y mouse lymphoma forward mutation assay, for inducing chromosomal aberrations, polyploidy or endoreduplication in Chinese hamster ovary cells, and in the in *vivo* mouse micronucleus assay (Butterworth et al., 2001). Further, a previous 18 month bioassay conducted with AQ administered to male and female B6C3F1 and (C57BL/6 x AKR) F1 mice reported no induction of cancer. Thus, it was somewhat unexpected that in a long-term study conducted by the National Toxicology Program (NTP) AQ-OX induced a weak to modest increase in tumors in the kidney and bladder of male and female F344/N rats and a strong increase in the livers of male and female B6C3F1 mice. In the studies reported in Butterworth et al. (2001) a sample of the AQ-OX used in the NTP bioassay was shown to be mutagenic in the Ames tester strains TA98, TA100 and TA1537. Addition of an S9 metabolic activation system decreased or eliminated the mutagenic activity.

In contrast, the purified NTP AQ-OX as well as the technical grade samples AQ-FC and AQ-DA were not mutagenic in the Ames test. The chemical structure of AQ does not suggest that the parent compound would be DNA reactive. Therefore, a mutagenic contaminant was present in the NTP bioassay sample that is either directly mutagenic or can be activated by

bacterial metabolism. Analytical studies showed that the primary contaminant 9-nitroanthracene (9-NA) was present in the NTP AQ-OX at a concentration of 1200 ppm but not in the purified material. The 9-NA and any other contaminants that might have been present in the NTP AQ-OX induced measurable mutagenicity at 9-NA concentrations as low as 0.15 µg/plate in tester strain TA98, indicating potent mutagenic activity (Butterworth et al., 2001).

An Ames test in Salmonella show positive results in TA100 and TA98 with and without metabolic activation (Zeiger, 1988). In another mutagenicity test in Salmonella was positive in TA98 and negative in TA100 with and without metabolism (Fu, 1985). The NTP bacterial reverse mutation assay positive results were found in TA98 and TA100 with and without S9 fraction (NTP, 2005).

Therefore, many of the reported genotoxicity and carcinogenicity results in the literature for AQ and AQ derivative compounds must be viewed with caution.

We have selected those tests which indicate the origin of anthraquinone, not being AQ-OX. Furthermore we would like to focus on AQ-FC. In addition we also present data on the analogue emodin. All data has been evaluated for its quality according to Klimisch categorization (Klimisch, 1997).

Results:

- Genetic toxicity in vitro: Bacterial reverse mutation assay:

1. - Butterworth BE, Mathre OB and Ballinger K (2001): Experimental results. Test method equivalent to OECD guideline 471. GLP study.

The substance AQ-FC did not show any mutagenic activity either with or without metabolic activation in the Ames tester strains (S. typhimurium TA1535, TA1537, TA98 and TA100 or in E. coli WP2uvrA).

2. - NTP (National Toxicology Programme) (2003). Experimental results. Test method equivalent to OECD guideline 471.

The substance anthraquinone (100% pure) did not show any mutagenic activity either with or without metabolic activation in the Ames tester strains used (S. typhimurium TA98, TA100 and TA102).

3. - NTP (National Toxicology Programme) (2004).Experimental results. Test method equivalent to OECD guideline 471.

The substance anthraquinone (Friedel-Crafts process) did not show any mutagenic activity either with or without metabolic activation in the Ames tester strains used (TA98 and TA100).

- Genetic toxicity in vitro: Chromosomal aberrations in mammalian cells:

NTP (National Toxicology Programme) (1991). Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 473.

Chromosome aberrations were induced in cultured CHO cells in the absence of S9 activation and in the presence of S9; the response observed without S9 was stronger than with S9.

- Genetic toxicity in vitro: Mammalian cell micronucleus test:

Kevekordes S, Spielberger J, Burghaus CM, Birkenkamp P, Zietz B, (2001): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 487.

The test substance did not reveal any micronuclei inducing activity in either human lymphocytes or in Hep-G2.

- Genetic toxicity in vitro: Gene mutation in mammalian cells:

1. - Müller SO, Eckert I, Lutz WK and Stopper H (1996): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 476.

The substance induced a moderate increase in mutant fraction (it was only tested without metabolic activation).

2. - Westendorf J, Marquardt H, Poginsky B, Dominiak M, Schmidt J and Marquardt (1990): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 476.

Treatment with the test substance did not result in an increase in the number of colonies resistant to 6 -TG. Exposure in suspension in the presence of liver homogenate was also negative.

3. - Bruggeman IM and van der Hoeven JCM (1984): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 476.

In the V79-HGPRT mutation assay, the test substance emodin was highly mutagenic without metabolic activation. However, the increase in the induction of mutation was observed at highly toxic concentrations. Furthermore, a low plating efficiency was observed in the negative and positive controls.

- Genetic toxicity in vivo: Mammalian erythrocyte Micronucleus test:

1. - Mengs U, Krumbiegel G and Völkner W (1997): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test according to OECD guideline 474.

There was no statistically significant enhancement in the frequency of micronucleated PCEs in comparison to the negative controls at both preparation intervals.

2. - NTP (National Toxicology Programme) (1992): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 474.

In peripheral blood samples from mice in the 14-week feed study, an increase in the frequency of micronucleated NCEs was seen in females, but not in males. The small increase in NCEs observed in the female mice was statistically significant (P=0.001), but no individual exposed group value differed significantly from the control value; the result in female mice was concluded to be weakly positive.

- Genetic toxicity in vivo: Mammalian bone marrow chromosome aberration test:

1. - NTP (National Toxicology Programme) (1993a): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 475.

No increases in the frequencies of micronucleated erythrocytes were observed in any of the treatment groups (only males were used).

2. - NTP (National Toxicology Programme) (1993b); Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD and MacGregor (2000): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 475.

No increases in the frequencies of micronucleated erythrocytes were observed in any of the treatment groups (males and females were used).

In vitro genotoxicity studies

Method	Results	Remarks	Reference
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) E. coli WP2 uvr A (met. act.: with and without) Test concentrations: 0, 30, 60, 125, 250, 500, 1000 and 2000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no ; negative controls valid: yes; positive controls valid: yes negative for E. coli WP2 uvr A(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no ; negative controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): anthraquinone**	Butterworth BE, Mathre OB and Ballinger K (2001)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) TA98, TA100 and TA102 (met. act.: with and without) Test concentrations: 0, 100, 333, 1000, 3333 and 10000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse	Evaluation of results: negative Test results: negative for TA98, TA100 and TA102(all strains/cell types tested) ; met. act.: with and without ; vehicle controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): anthraquinone**	NTP (National Toxicology Programme) (2003)

Method	Results	Remarks	Reference
Mutation Assay)			
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) TA98 and TA100 (met. act.: with and without) Test concentrations: 0, 30, 100, 300, 1000, 3000 and 10000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for TA98 and TA100(all strains/cell types tested) ; met. act.: with and without ; vehicle controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): anthraquinone**	NTP (National Toxicology Programme) (2004)
mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: without) Test concentrations: 37, 55.5, 74 and 111 µM equivalent or similar to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	Evaluation of results: the compound induced a moderate increase in mutant fraction (it was only tested without metabolic activation) Test results: the compound induced a moderate increase in mutant fraction (it was only tested without metabolic activation) for mouse lymphoma L5178Y cells ; met. act.: without ; cytotoxicity: yes (at highest dose) ; vehicle controls valid: yes; positive controls valid: positive control only valid in the first experiment	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	Müller SO, Eckert I, Lutz WK and Stopper H (1996)

Method	Results	Remarks	Reference
mammalian cell gene mutation assay (gene mutation) Chinese hamster lung fibroblasts (V79) (met. act.: with and without) Test concentrations: Without metabolic activation: 0, 10, 20, 25 and 30 mg/l With metabolic activation: 0, 25, 50 and 100 mg/l equivalent or similar to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	Evaluation of results: negative Test results: negative for Chinese hamster lung fibroblasts (V79) ; met. act.: with and without ; cytotoxicity: yes (at concentrations of 20 mg/l and higher without metabolic activation) ; vehicle controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	Bruggeman IM and van der Hoeven JCM (1984)
in vitro mammalian chromosome aberration test (chromosome aberration) Chinese hamster Ovary (CHO) (met. act.: with and without) Test concentrations: Without metabolic activation: 10, 15 and 20 µg/mL With metabolic activation: 100, 150 and 200 µg/mL	Evaluation of results: positive without metabolic activation weakly positive with metabolic activation Test results: positive for Chinese hamster Ovary (CHO) ; met. act.: without ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes weakly positive for Chinese hamster Ovary (CHO) ; met. act.:	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	NTP (National Toxicology Programme) (1991)

Method	Results	Remarks	Reference
equivalent or similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	with ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes		
in vitro mammalian cell micronucleus test (chromosome aberration) lymphocytes: human peripheral lymphocyte cultures (met. act.: with and without) human hepatoma cell line Hep-G2 Test concentrations: 0, 5, 10, 50, 100, 500 and 1000 µM equivalent or similar to OECD Guideline 487	Evaluation of results: negative Test results: negative for human peripheral lymphocyte cultures ; met. act.: with and without ; cytotoxicity: yes (at 1000 µM without metabolic activation and at 500 µM with metabolic activation) ; negative controls valid: yes; positive controls valid: yes negative for human hepatoma cell line Hep-G2 ; met. act.: with ; cytotoxicity: yes (at 1000 µM) ; negative controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	Kevekordes S, Spielberger J, Burghaus CM, Birkenkamp P, Zietz B, (2001)

In vivo genotoxicity studies

Method	Results	Remarks	Reference
micronucleus assay (chromosome aberration) mouse (NMRI) male/female oral: gavage 2000 mg/kg (actual ingested) OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Evaluation of results: negative Test results: Genotoxicity: negative (male/female); toxicity: no effects ; vehicle controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	Mengs U, Krumbiegel G and Völkner W (1997)
micronucleus assay (chromosome aberration) rat (Fischer 344) male intraperitoneal 125, 250 and 500 mg/kg (injected) equivalent or similar to OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test)	Evaluation of results: negative Test results: Genotoxicity: negative (male) ; vehicle controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	NTP (National Toxicology Programme) (1992)
micronucleus assay (chromosome	Evaluation of results: negative	2 (reliable with restrictions)	NTP (National Toxicology

Method	Results	Remarks	Reference
aberration) mouse (B6C3F1) male/female intraperitoneal 125, 250 and 500 mg/kg (injected) equivalent or similar to OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test)	Test results: Genotoxicity: negative (male/female) ; vehicle controls valid: yes; positive controls valid: yes	key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	Programme) (1993a)
micronucleus assay (chromosome aberration) mouse (B6C3F1) male/female oral: feed 312, 625, 1250, 2500 and 5000 ppm (nominal in diet) equivalent or similar to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Evaluation of results: negative in males and weakly positive in females Test results: Genotoxicity: negative (male/female) ; negative controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	NTP (National Toxicology Programme) (1993b) Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD and MacGregor (2000)

Carcinogenicity

NTP carcinogenicity assay (NTP, 2005) has not been included in this summary due to the plausible contamination of test sample. Tests in analogues have been selected for this section. Evidences of non effects for carcinogenicity of anthraquinone non AQ-OX can be seen from read-across with the analogue emodin.

Results:

NTP (National Toxicology Programme) (2001): Read-across from experimental data on the analogue CAS No. 518-82-1 (emodin). Test method equivalent to OECD guideline 451. GLP study. Tested in rats and mice.

There was no evidence of carcinogenic activity of emodin in male rats. There was equivocal evidence of carcinogenic activity in female rats.

There was equivocal evidence of carcinogenic activity in male mice. There was no evidence of carcinogenic activity in female mice.

Results rats: HISTOPATHOLOGY: NEOPLASTIC:

Pituitary Gland: The incidences of adenoma of the pars distalis in female rats were significantly increased in the 830 and 2,500 ppm groups compared to the control group. However, the incidences in the exposed groups were similar to the historical control incidence of 443/893 (49.5% ± 12.0%; range, 33%-72%). The significantly increased incidences appear to be related to the unusually low incidence in the concurrent control group (31%), which is just below the range in historical controls, rather than an effect of exposure to emodin. Additionally, there was no increase in the incidence of hyperplasia in females and no evidence of an increase in the incidence of proliferative lesions in male rats. For these reasons, the increased incidences of pars distalis adenomas in females were not considered to be exposure related.

Nose: Squamous cell carcinomas were noted in the nasal cavity of two male rats exposed to 2,500 ppm. The carcinomas were located at the first and second levels of the nasal cavity. In one rat, the carcinomas appeared to originate from the nasal septa, while in the other animal, the carcinoma seemed to originate from the maxilloturbinate and the base of the nasal cavity. In both cases, the carcinomas were well differentiated, showing keratinization and formation of typical keratin pearls. Extensive invasion into the surrounding underlying tissue was evident, as was superficial secondary inflammation. Squamous cell carcinomas are rare in F344/N rats; the historical control range for squamous cell carcinomas in feed studies is 0% to 2%. However, no other lesions were present in the nose. The absence of preneoplastic lesions or other chemical-related lesions is consistent with an absence of response to chemical exposure in the nose. Therefore, the two squamous cell carcinomas were considered unrelated to emodin exposure.

Mononuclear Cell Leukemia: There were negative trends in the incidences of mononuclear cell leukemia in male and female rats and the incidences in the 2,500 ppm groups were significantly less than those in the controls. The decreased incidences were particularly evident in the liver, spleen, and bone marrow, which are among the organs most frequently

affected by mononuclear cell leukemia. The incidence in females exposed to 2,500 ppm was below the historical control range; the incidence in 2,500 ppm males was at the lower end of the historical control range.

UNCERTAIN FINDINGS:

Zymbal's Gland: Zymbal's gland carcinomas were noted in three female rats exposed to 2,500 ppm. Two of the carcinomas were well differentiated, containing neoplastic sebaceous cells and squamous epithelium with keratin; the third carcinoma consisted of poorly differentiated, highly invasive neoplastic islands. This range of morphology is typically described in spontaneous neoplasms in rats (Seely, 1991). Single cases of the same neoplasm were also seen in one male rat exposed to 280 ppm and in one 2,500 ppm male rat. The incidence of carcinoma in 2,500 ppm females exceeded the range in historical controls in 2-year NTP feed studies. The historical incidence for Zymbal's gland carcinoma in untreated male rats is 9/904 (1.0% ± 1.2%; range, 0%-4%).

Results mice: HISTOPATHOLOGY: NEOPLASTIC: None

All Organs: A decrease in the incidence of malignant lymphoma was noted in male mice receiving 625 ppm. This incidence was at the lower end of the historical control range.

Lung: The incidences of alveolar/bronchiolar carcinoma were decreased in groups of exposed male mice. This apparent decrease was primarily due to the high incidences in the control and 160 ppm groups; the incidences in these groups were outside the current historical control range. The incidences of alveolar epithelial hyperplasia were not decreased and no decreases were observed in female mice. This marginal decrease in the incidences of alveolar/bronchiolar carcinoma was not considered to be treatment related.

UNCERTAIN FINDINGS:

Kidney: Although not significantly increased, there were rare renal tubule neoplasms in the kidney of males. The incidence of adenoma or carcinoma (combined) in males exposed to 312 ppm exceeded the historical control range. Renal tubule hyperplasia, adenoma, and carcinoma are considered to represent a morphologic and biologic continuum of proliferative lesions; however, there was no significant increase in the incidences of hyperplasia. Initially, a single H&E-stained section of each kidney was prepared and evaluated microscopically. In this study, additional sections of the remaining formalin-fixed kidneys were taken at 0.5-mm intervals, resulting in approximately four additional sections per animal. A single additional adenoma was identified in a 625 ppm male. Hyperplasia was generally a focal, minimal to moderate lesion consisting of tubules that were dilated two to five times the normal diameter and were lined by increased numbers of tubule epithelial cells that partially or totally filled the tubule lumen. Usually, it was associated with dilatation of the tubule. Cells within the hyperplastic lesions varied slightly in size but otherwise appeared similar to normal tubule epithelial cells. Renal tubule adenomas were larger, discrete lesions, generally ranging from five tubules in diameter up to approximately 1 cm. Cells within adenomas consisted of

relatively normal-appearing tubule epithelial cells that sometimes formed solid masses of multiple clusters of cells. Renal tubule carcinomas were larger than adenomas, associated with hemorrhage, necrosis or local invasion, and cellular anaplasia or atypia.

Carcinogenicity studies:

Method	Results	Remarks	Reference
rat (Fischer 344) male/female oral: feed 0, 280, 830, or 2,500 ppm emodin (equivalent to average daily doses of approximately 110, 320, or 1,000 mg/kg to males and 120, 370, or 1,100 mg/kg to females) (nominal in diet) Exposure: 105 weeks (Daily) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	NOAEL (toxicity): > 1100 mg/kg bw/day (female) based on: test mat. (No adverse effects observed) no NOAEL identified (toxicity): (male) based on: test mat. (Statistically significant decreases were observed in absolute and relative liver weights at the 12-month interim evaluation in males from the treatment groups 280, 830 and 2500 ppm.) Neoplastic effects: no effects	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	NTP (National Toxicology Programme) (2001)
mouse (B6C3F1) male/female oral: feed Males: 0, 160, 312, or 625 ppm emodin (equivalent to average daily doses of approximately 15, 35, or 70 mg/kg) (nominal in diet) Females: 0, 312, 625, or 1,250 ppm emodin (equivalent to average daily doses of approximately 30, 60, or 120 mg/kg) (nominal in diet) Exposure: 105 weeks (Daily) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	NOAEL (toxicity): > 70 mg/kg bw/day (male) based on: test mat. (No adverse effects observed) NOAEL (toxicity): > 120 mg/kg bw/day (female) based on: test mat. (No adverse effects observed) Neoplastic effects: no effects	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3,8-trihydroxy-6-methylanthraquinone**	NTP (National Toxicology Programme) (2001)

6.- DISCUSSION

AQ has been extensively studied for genotoxicity being non mutagen in Ames tests performed by different authors (Brown and Brown, 1976; Anderson and Styles, 1978; Gibson *et al.*, 1978; Salamone *et al.*, 1979; Sakai *et al.*, 1985; Tikkanen *et al.*, 1983; National Cancer Institute, 1987; Bulleworth 2001; NTP 2003; NTP 2004).

The analogues Aloe-emodin (1,8-dihydroxy-3-(hydroxymethyl)-anthraquinone) and emodin (1,3,8-trihydroxy-6-methylanthraquinone) are active components contained in the root and rhizome of *Rheum palmatum L*. Both substances have been reported to be potent anticancer agent for the treatment of multiple cancers (Hong-Zin Lee , 2001; Muto, 2007; Srinivas 2007). Genotoxicity test have been carried out on emodin by different authors with negative output in mammalian cells assay (Muller 1996; Bruggeman 1984) , chromosamal aberrations in vitro (NTP 1991; Kevekordes 2001) and in vivo (Mengs 1997; NTP 1992; NTP 1993a; NTP 1993b, Witt 2000) and also carcinogenicity test in rat and mice (NTP 2001).

Butterworth et al. concluded that impurities are responsible for positive output in NTP assay "Literature reports of mutagenic activity for 1-OH-AQ and 2-OH-AQ in bacteria with S9. Weak activity and limited production of the 1-OH-AQ and 2-OH-AQ metabolites are possible reasons that AQ fails to exhibit activity in numerous genotoxicity assays. 9-NA was mutagenic in tester strains TA98 and TA100 minus S9. This pattern of activity is consistent with that seen with the contaminated AQ-OX used in the NTP bioassay. Quantitative mutagenicity and carcinogenicity potency estimates indicate that it is plausible that the contaminants alone in the NTP AQ-OX bioassay could have been responsible for all of the observed carcinogenic activity". (Butterworth, 2004).

Same results are extracted from test performed with metabolites of above mentioned analogues (Mueller, 1998). Although hydroxylated metabolites show a weak activity in vitro, are not showing these effects in vivo. This is likely to be due to the rapid clearance.

Same pattern is shown by analogue emodin and its metabolites which supports lack of genotoxicity/carcinogenicity potential of this group of chemicals.

7.- CONCLUSION

There are clear evidences on the different genotoxic and carcinogenicity effects of anthraquinone depending on manufacture method. It has been demonstrated by different authors (Butterworth, 2001; Butterworth, 2004) that NTP carcinogenicity assay was performed with a contaminated sample with 9-nitroanthraquinone, a potent carcinogen as well as other polycyclic aromatic compounds.

Therefore it is considered demonstrated the different toxicological profile of AQ-OX and AQ-FC, this last one being non genotoxic and non carcinogenic.

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