

## COMMENTS TO PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Based on Regulation (EC) N° 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name	Butanone Oxime
EC number	202-496-6
CAS Number	96-29-7
Index Number	616-014-00-0

### Comments on carcinogenicity classification proposal.

After evaluation of the toxicological available data on butanone oxime, we concluded that there are some reasons for withdrawal of the classification proposal. These are:

#### 1.- Quality of carcinogenicity test

The proposal for classification of butanone oxime as carcinogen cat. 1, is solely based on Newton et al. (2001) assays. These studies show several deficiencies:

- ✓ Mode of dosing: not guidance mode (whole body exposure= extremely over-exposing animals)
- ✓ Butanone Oxime is volatilized, forcing it in a way that should be investigated if other chemicals species are formed, and present in the air.
- ✓ Doses (extremely high). Highest dose was 1350mg/m<sup>3</sup>. This is the level where they detect the tumors after 28/ 18 months.

In sub-chronic test (inhalatory) we can see effects at 36 mg/m<sup>3</sup>:

specie	Duration	NOEAC	LOAEC	Seen effect
mice	<b>90 days</b>	10,8	<b>36</b>	Olfactorium epithelium degeneration

However, after 18 months, in carcinogenicity assay they found **no effect** at 53 mg/m<sup>3</sup>

specie	Duration	NOEAC	Seen effect
mice	<b>18 months</b>	<b>53</b>	Olfactorium epithelium degeneration Hepatocelular adenomas

✓ Mortality (very high, including controls)

OECD Guideline n° 116 establishes: *“Termination of the study should be considered when the number of survivors in the lower dose groups or the control group falls below 25 per cent, considering the survival of each sex separately. The US EPA Health Effects Test Guidelines 870.4200 (US EPA, 1998b) specify that survival in any group should not fall below 50% at 15 months in the case of mice and 18 months in the case of rats, or below 25% at 18 and 24 months respectively”*

According to table 1 of Newton et al. publication, survival rate is lower than 50% in all dose groups and control of male rats and higher dose and control of male mice.

These low figures would compromise the statistical part of the study.

✓ No tumors are seen in females

✓ Liver tumors appears in last period of life in males

✓ For some tumors in males and all for females incidence is lower than in controls, with  $P < 0.05$ .

## **2.- Mode of action- non genotoxic substance**

There is a complete set of available data on genotoxicity assays in vitro and in vivo, all of them being negative. Therefore, the substance is clearly NOT genotoxic. Epigenetic factors contribute to the hypothetically carcinogenic effect.

The authors described *“Changes were also seen in the liver, appeared related to hepatotoxicity and occurred with greater incidence primarily in animals exposed to 75 and 374 ppm. These changes consisted of centrilobular hepatocellular hypertrophy and necrosis and were considered to be treatment-related.(...) The treatment related findings in the liver included hepatocellular carcinoma in male rats in the 374 ppm group and increase of hepatocellular adenoma in males of thr 374 and 75 ppm groups (...).*

The authors stated *“the increased liver and spleen weights with MEKO exposure are related in part to the increased red cell destruction (hemosiderin deposition and extramedullary hematopoiesis). The anaemia is considered to be compensatory....”*

The substance causes haemolytic anaemia, with haematological parameters being affected at 374 and 75 ppm. A compensatory effects/adaptation is seen. Rat appears to be more sensitive to these effects than mice.

All these findings are very much concordant with **well known hemosiderin deposition effects**. Iron can accumulate in the liver in a variety of conditions, including congenital, systemic iron-loading conditions (hereditary hemochromatosis), conditions associated with systemic macrophage iron accumulation (transfusions, **haemolytic** conditions, **anaemia** of chronic disease, etc), in some hepatitides (hepatitis C, alcoholic liver disease, porphyria cutanea tarda), and liver-specific iron accumulation of uncertain pathogenesis in cirrhosis. Iron accumulation in the liver is significant and, if so, the nature of the disease that leads to the accumulation might differ.

This haemolytic response is translated into the accumulation of iron in both liver hepatocytes and Kupffer cells. The Kupffer cell response to this insult is two-fold: (1) the production of oxidative species-through both Kupffer cell activation and through the Fenton reaction involving iron and (2) the production of cytokines. The induction of reactive oxygen species can, if not scavenged, produce oxidative DNA damage, as well as increase cell growth through modulation of gene expression.

The occurrence of liver hemangiosarcomas is linked to oxidative damage subsequent to red blood cell haemolysis and iron deposition in this organ. This is a nonlinear mode of action and may be dependent on threshold events such as chemical-induced haemolytic effects.

Most chemicals could demonstrate increased tumour incidence rates at the maximum-tolerated dose (MTD) in rodents. With an MTD that may produce a difference (up to 10%) in weight gain between treated and control animals, there quite possibly is cytotoxicity at the MTD. Increased carcinogenicity would be expected from increased opportunities for mutagenic activity during regenerative cell replication to compensate for cytotoxicity. Since it appears that almost all chemicals tested adequately at the MTD will demonstrate carcinogenicity, it is tempting to surmise that this is due in large part to one or more nearly universal modes of action, such as, regenerative cell replication at the MTD rather than due to some unique carcinogenic property of a chemical (Haseman and Seilkop, 1992)<sup>1</sup>

**Therefore, there is a threshold for the toxicity effects, being the haemolytic anaemia the initiating event, and the rest of cascade of secondary effects in excess toxicity, to be seen until compensation appears.**

### **3.- CLP principles are not met; for category 1B carcinogen :**

It is stated that: "...causal relationship has been established between the agent and an increased incidence of malignant neoplasm's or of an appropriate combination of benign and malign neoplasm in:

- a) Two or more species of animals or in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols
- b) In both sexes of a single species
- c) Occurrence of malignant neoplasm to an unusual degree with regard to the incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites

For the first condition (a):

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<sup>1</sup> Haseman JK, Seilkop SK. (1992) An examination of the association between maximum-tolerated dose and carcinogenicity in 326 long-term studies in rats and mice. *Fundam Appl Toxicol.* 1992 Aug;19(2):207-13.

- *Two or more species of animals or*  
No tumorigenic effects have been detected in mice
- *in two or more independent studies in one species carried out at different times or*  
This is one study done by the same author and at the same time
- *in different laboratories or under different protocols*  
both studies are done by same laboratory under same guideline.

For the second condition (b)

- *In both sexes of a single species*  
No effects have been seen in female mice “ time-to-tumour analysis showed MEKO not related to benign or malignant tumour production or to time to tumour in female CD-1 mice”

For the third condition (c):

- *Occurrence of malignant neoplasm to an unusual degree with regard to the incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites*

Incidence is not statistically significant

#### **4.- Risk assessment considerations**

The current exposure limit at workplace has been set as 0.3 ppm (AGW: 1 mg/m<sup>3</sup> (0,3 ppm)). The NOAEC for inhalatory effects is 54 mg/m<sup>3</sup>

Therefore, an assessment factor of 54 is *de facto* in place for workers inhalatory exposure. This is considered as very conservative in terms of risk assessment. The observed effects in rodents appeared at exposure levels which are not foreseen due to AGW value in place.

Therefore, the current classification and the exposure limit at workplace are demonstrated to be restrictive enough to protect human health.

## **CONCLUSIONS**

1. There are no known non-genotoxic carcinogens to be liver tumorigenic- it is well described that this is a typical false positive.
2. Treatment-related effects are seen only at toxic doses, concomitant with haemolitical anemia. Those are also concordant with the specie-specificity for anemia and the time to disappear tumorigenic effects.
3. In absence of toxicity excess, no tumours are seen in any specie or gender.

4. CLP principles are not met; for category 1B carcinogen :

**Taking into account all available data on butanone oxime, we believe that no additional classification on carcinogenicity hazard is justified neither from the CLP principles nor scientifically on the light of the available data.**