

**Committee for Risk Assessment**  
**RAC**

**Opinion**

on the specific target organ toxicity of  
**2-benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)**

**EC number: 223-346-6**  
**CAS number: 3846-71-7**

and

**2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)**

**EC number: 247-384-8**  
**CAS number: 25973-55-1**

ECHA/RAC/A77-O-0000003444-77-02/F

**Adopted**

**10 June 2013**

**OPINION OF THE COMMITTEE FOR RISK ASSESSMENT  
ON THE SPECIFIC TARGET ORGAN TOXICITY OF**

**2-BENZOTRIAZOL-2-YL-4,6-DI-TERT-BUTYLPHENOL (UV-320)  
AND  
2-(2H-BENZOTRIAZOL-2-YL)-4,6-DITERTPENTYLPHENOL (UV-328)**

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (the REACH Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on repeated dose toxicity of UV-320 and UV-328.

**I      PROCESS FOR ADOPTION OF THE OPINION**

In the mandate of 4 April 2013 attached as Annex 1, the Executive Director of ECHA requested the RAC to provide an opinion on the specific target organ toxicity of UV-320 and UV-328 taking into account the information provided in Annex XV dossiers for the identification of substances of very high concern (SVHC) and the comments submitted on the 'T' hazard during the public consultation on these dossiers. It should be noted that this was not intended as a request for an opinion on harmonised classification and labelling as such; it was solely intended to provide advice to the Member State Committee (MSC) in this specific case.

The Annex XV SVHC dossiers were made publicly available at: <http://echa.europa.eu/proposals-to-identify-substances-of-very-high-concern> on **4 March 2013**. Parties concerned and MSCAs were invited to submit comments and contributions by **18 April 2013**.

**II      ADOPTION OF THE OPINION OF THE RAC**

Rapporteur, appointed by the RAC:                    **Boguslaw Baranski**

The RAC opinion was adopted on 10 June 2013.

The RAC opinion was adopted by consensus.

**III      OPINION OF THE RAC**

The RAC has formulated its opinion on:

- a) whether the information provided in the Annex XV SVHC dossiers is sufficient to develop an opinion of a similar robustness to a CLH opinion,
- b) whether the information provided shows that the substance meets the criteria for classification for specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) under CLP.

After examination of the information provided in the SVHC Annex XV dossiers and the comments related to specific target organ toxicity following repeated exposure raised during the public consultation, the RAC agreed that this information shows that the substances UV-320 and UV-328 both meet the criteria for classification **as STOT RE 2** as defined in the CLP Regulation (EC) 1272/2008.

#### **IV SCIENTIFIC GROUNDS FOR THE OPINION**

##### **RAC evaluation of specific target organ toxicity (CLP) – repeated exposure (STOT RE)**

##### **2-BENZOTRIAZOL-2-YL-4,6-DI-TERT-BUTYLPHENOL (UV-320)**

##### **Summary of the Dossier submitter's proposal**

The dossier submitter (DS, German competent authority) proposed that UV-320 be classified for "specific target organ toxicity – repeat exposure" in sub-category 1 (STOT RE 1) and therefore be considered as toxic, complying with the 'T' criterion for PBT definition under the REACH Regulation. UV-320 is not registered under REACH.

The basis for the STOT RE 1 classification is derived from a sub-acute (28-day) toxicity study in rats (Hirata-Koizumi *et al.*, 2007). Repeated oral (gavage) administration of UV-320 caused toxicity in several organs, in particular in the liver. Briefly, microscopic examination revealed hypertrophy of hepatocytes starting from 0.5 mg/kg bw/d. At higher dose levels (i.e. from 2.5 mg/kg bw/d), males showed hepatocellular vacuolar degeneration and focal necrosis. Bile duct proliferation was also observed from 0.5 mg/kg bw/d.

The DS concluded that because the LOAEL is < 10 mg/kg bw/d, the subcategory STOT RE 1 is fulfilled, in line with CLP classification criteria. In conclusion, based on the provisions of Annex XIII, section 1.1.3 (c) of the REACH Regulation, the DS also concluded that UV-320 meets the 'T' criterion.

##### **Comments received during public consultation**

Some comments provided support to the DS proposal on the identification of the substance as an SVHC. Other comments considered the available vPvB/PBT data relatively weak. One MS competent authority (CA) which did not specifically address STOT RE but emphasised the lack of robust information to conclude on the PBT/vPvB status.

Regarding STOT RE, a further MSCA requested additional details to properly assess the classification proposal. In particular, they asked for clarifications on whether the adverse toxicity effects reported were increased in a dose-related and statistically significant way. They also requested clarification from the DS regarding the choice of the key study and on the comparison with the CLP criteria.

The a third MSCA agreed with the DS on STOT RE 1 on the basis of severe toxicity occurring in several target organs (liver, heart, spleen) at a dose fulfilling the CLP criteria for STOT RE 1.

##### **Assessment and comparison with the classification criteria**

The RAC considers the information provided in the SVHC Annex XV dossier not to be sufficient to develop an opinion of a similar robustness to a CLH opinion, because the severity of the effects were not described in sufficient details and the effects were not analysed taking into account the guidance values (in mg/kg

bw/day) provided in tables 3.9.2 and 3.9.3 of Annex I to the CLP regulation. In addition, the severity and significance of the effects were not compared with criteria provided in sections 3.9.2.7 and 3.9.2.8 of Annex I to the CLP Regulation. The references for two of the three studies considered in the SVHC Annex XV dossier were provided during public consultation.

Therefore, the results of studies considered by the DS (listed below and in the reference list) were summarized in this opinion and compared with classification criteria:

1. Hirata-Koizumi M, Watari N, Mukai D, Imai T, Hirose A, Kamata E, Ema M (2007). A 28-Day Repeated Dose Toxicity Study of Ultraviolet Absorber 2-(2'-Hydroxy-3',5'-di-*tert*butylphenyl) benzotriazole in Rats. *Drug and Chemical Toxicology*, 30: 327-341
2. Hirata-Koizumi M, Matsuyama T, Imai T, Hirose A, Kamata E, Ema M (2008a). Gonadal Influence on the Toxicity of 2-(2'-Hydroxy-3',5'-di-*tert*-butylphenyl) benzotriazole in Rats. *Drug and Chemical Toxicology*, 31: 115-126.
3. Hirata-Koizumi M, Ogata H, Imai T, Hirose A, Kamata E, Ema M (2008b). A 52-Week Repeated Dose Toxicity Study of Ultraviolet Absorber 2-(2'-hydroxy-3',5'-di-*tert*butyl-phenyl) benzotriazole in Rats. *Drug and Chemical Toxicology* 31: 81-96.

#### CLP classification criteria for repeated target organ toxicity (STOT RE)

According to the CLP Regulation (section 3.9.2.) substances are classified as specific target organ toxicants following repeated exposure (STOT RE) by the use of expert judgement on the basis of the weight of all available evidence.

Substances are classified in Category STOT RE 1 on the basis of:

- reliable and good quality evidence from human cases or epidemiological studies; or
- observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Substances are classified in Category STOT RE 2 on the basis of:

- observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. The classification is applicable when significant toxic effects are observed in a 90-day repeated dose study conducted in experimental animals at or below the guidance values provided in table 3.9.2 for Category 1 and table 3.9.3 of CLP Regulation for category 2. In case of oral exposure (rat) they are either  $\leq 10$  mg/kg/day for Category 1 or they are in a range between 10 and 100 mg/bw /day for Category 2

For a 28-day study this guidance value is increased by a factor of three.

As defined in section 3.9.2.7.3 of the CLP Regulation, all available evidence, and its relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:

- (a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver);

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

It should be noted that as defined in point 3.9.2.8.1. of Annex I of CLP there are some effects in humans and/or animals that do not justify classification. Such effects include, but are not limited to:

(a) clinical observations or small changes in bodyweight gain, food consumption or water intake that have toxicological importance but that do not, by themselves, indicate 'significant' toxicity;

(b) small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance;

(c) changes in organ weights with no evidence of organ dysfunction;

(d) adaptive responses that are not considered toxicologically relevant;

(e) substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, shall not justify classification.

#### Summary of target organ toxicity induced by UV-320 after repeated exposure

In the 28-day study of Hirata-Koizumi *et al.* (2007) females and male rats were administered UV-320 (2-benzotriazol-2-yl-4,6-di-tert-butylphenol) by gavage at a dose of 0 (vehicle: corn oil), 0.5, 2.5, 12.5, or 62.5 mg/kg bw/day for 28 days. This study was performed in compliance with the Test Guideline of the Japanese Chemical Control Act and in accordance with GLP. The initial numbers of rats were 10/sex in the control and the highest dose group, and 5/sex in other dose groups. The day after the last dosing, 5 males and 5 females from each group were euthanized for the assessment of hematology, blood biochemistry, organ weights, and macroscopic and microscopic findings.

The exposure to UV-320 did not result in treatment-related mortality or clinical signs of toxicity in any groups. There were also no significant changes in body weight, but a significant increase in food consumption was noted on dosing days 14 and 21 in males and on dosing days 21 and 27 in females at 62.5 mg/kg. No dose-related changes were found in the findings of urinalysis.

At the completion of dosing, a decrease in red blood cells, hemoglobin, and hematocrit was noted only in males at 2.5 mg/kg and more, but not in female rats. A small, although statistically significant reduction of red blood cells, hemoglobin, and hematocrit (amounting to 9%, 10.5% and 8.1% of the mean control values, respectively) was seen at the highest dose of 62.5 mg/kg bw/day. The degree of anemia at the highest dose does not meet the criteria of significant adverse effects (e.g. reduction in Hb at  $\geq 20\%$ ) as defined in section 3.9.2.5.2 of the Guidance on the Application of the CLP Criteria for STOT RE classification.

Blood biochemical examination revealed statistically significant dose-dependent increases in:

- albumin level increased significantly from 3.78 g/dL in control males to 4.43 mg/dL and 4.40 mg/dL in rats exposed to 12.5 mg/kg and 62.5 mg/kg, no significant increase of albumin level was noted in females
- albumin/globulin ratio in males at 0.5 mg/kg and more (from 1.85 in controls to 3.05 in the 62.5 mg/kg group; in females increase in albumin/globulin ratio was noted only in the 62.5 mg/kg group; from 2.04 in controls to 4.21 in the 62.5 mg/kg group
- levels of glucose increased from 122 mg/dL in control males to 170, 170 and 156 mg/dL in male rats exposed to 2.5 mg/kg, 12.5 mg/kg and 62.5 mg/kg, respectively and from 110 mg/dL in control females to 151 mg/dL in female rats exposed to 62.5 mg/kg,
- urea nitrogen (BUN) level increased significantly only in males exposed to 62.5 mg/kg –from 13.0 mg/L to 17.2 mg/L
- aspartate aminotransferase (AST) level increased significantly only in males exposed to 62.5 mg/kg –from 72 U/L to 115 U/L
- alanine aminotransferase (ALT) level increased significantly in male rats exposed to 62.5 mg/kg –from 30 U/L to 48 U/L and females – from 21 U/L to 33 U/L
- total cholesterol and triglyceride levels were increased only in females exposed at 62.5 mg/kg; cholesterol from 49 mg/dl in control females to 84 mg/dl in exposed females and triglyceride from 12.3 mg/dl in control females to 31.9 mg/dl in exposed females

From the changes described above, increased serum levels of AST, ALT, BUN, total cholesterol, triglyceride and glucose induced by UV-320 at the dose of 62.5 mg/kg are not by themselves considered sufficiently adverse effects. However, in conjunction with histopathological changes in the liver, they are considered as consistent and significant adverse effects which meet criterion of specific target organ toxicity-repeated exposure as set out in section 3.9.2.7.3 of CLP.

At necropsy, absolute liver weight was significantly increased in males from 9.4 g in control group to 17.1 g, 21.6 g and 24.5 g in males exposed to 2.5, 12.5 and 62.5 mg/kg respectively. In females there was a significant increase in absolute liver weight from 6.4 g in control group to 8.7 g and 12.4 g in females exposed to 12.5 and 62.5 mg/kg respectively. In the highest dose group (62.5 mg/kg), there was also a significant increase in absolute and relative kidney weight in males and in absolute heart weight in females. No test substance-related significant effects were detected in other organs.

During histochemical analysis test substance-related effects were observed in the liver, heart, kidneys, thyroids and spleen.

In the liver:

- hypertrophy of hepatocytes was observed in 3, 5, 5 and 5 out of 5 examined male rats exposed to 0.5, 2.5, 12.5 and 62.5 mg/kg, respectively and in 5 out of 5 examined female rats at 12.5 and 62.5 mg/kg; respectively
- hepatocellular fatty change was mostly observed in control animals: in males it was observed in 5 out of 5 control animals, while this change was not observed in exposed males; in females a hepatocellular fatty change was observed in 5 out of 5 examined control females and in females exposed to 0.5 mg/kg and 2.5 mg/kg, in 3 and in 0 females out 5 examined females exposed to 12.5 and 62.5 mg/kg; respectively

- vacuolar degeneration of hepatocytes was observed in 5 out of 5 examined male rats exposed to 2.5, 12.5 and 62.5 mg/kg, respectively and in 2 out of 5 examined female rats at 62.5 mg/kg
- increased mitosis of hepatocytes was observed in 4 out of 5 examined male rats exposed to 62.5 mg/kg, and in females in 1 and 2 out of 5 examined female rats exposed to 12.5 and 62.5 mg/kg; respectively
- focal necrosis was observed in 1, 2 and 4 out of 5 examined male rats exposed to 2.5, 12.5 and 62.5 mg/kg, respectively
- hepatocellular pigmentation and/or cytoplasmic inclusion bodies was observed in 1 out of 5 examined males exposed to 62.5 mg/kg
- bile duct proliferation was observed in 1, 1, 4 and 4 out of 5 examined male rats exposed to 0.5, 2.5, 12.5 and 62.5 mg/kg, respectively and in 1 out of 5 examined female rats at 62.5 mg/kg;
- the above histopathological changes were reported as slight for males and females. Except for increased mitosis, the effects were still present at the completion of the 14-day recovery period at the dose of 62.5 mg/kg.

#### In the heart:

- slight cell infiltration was observed in 5, 4 and 4 out of 5 examined male rats exposed to 2.5, 12.5 and 62.5 mg/kg, respectively and in 1 and 1 out of 5 examined female rats exposed to 0.5 and 62.5 mg/kg, respectively
- slight hypertrophy of the myocardium was observed in 3 and 4 out of 5 examined male rats exposed to 12.5 and 62.5 mg/kg, respectively and in 1 and 3 out of 5 examined female rats exposed to 12.5 mg/kg and 62.5 mg/kg, respectively
- slight degeneration of the myocardium was observed in 5 out of 5 examined male rats exposed to 12.5 and 62.5 mg/kg, and in 3 and 5 out of 5 examined female rats exposed to 12.5 mg/kg and 62.5 mg/kg, respectively
- except hypertrophy, the above histopathological changes were also reported in males only at the completion of the 14-day recovery period at the dose of 62.5 mg/kg.

#### In the kidneys:

- slight hypertrophy of the tubular epithelium was observed in the kidneys of 2 and 5 out of 5 examined male rats exposed to 12.5 and 62.5 mg/kg, respectively, and in 2 out of 5 examined females at 62.5 mg/kg
- slight to moderate basophilic tubules were observed in the kidneys of 2, 3, 4, 3, 5 out of 5 examined male rats exposed to 0, 0.5, 2.5, 12.5 and 62.5 mg/kg, respectively. The severity was moderate for 2 out of 5 male rats dosed at 62.5 mg/kg. Basophilic tubules were observed (with no clear dose-response or increased severity) in the kidneys of 1, 2, 2, 0, 3 out of 5 examined female rats exposed to 0, 0.5, 2.5, 12.5 and 62.5 mg/kg, respectively
- the above changes were completely recovered at the completion of the 14-day recovery period at the dose of 62.5 mg/kg.

#### In the thyroids:

- slight diffuse follicular cell hyperplasia was observed in 2 males and in 2 females out of 5 examined animals at 62.5 mg/kg

- diffuse hyperplasia was reported in 3 out of 5 males at the completion of the 14-day recovery period.

In the spleen:

- slight extramedullary hematopoiesis was observed in 3, 2 and 2 out of 5 examined male rats exposed to 2.5, 12.5 and 62.5 mg/kg, respectively and in 1 out of 5 examined female rats exposed to 0.5 mg/kg/day.
- after completion of the 14-day recovery period the slight extramedullary hematopoiesis was still seen in 3 out of 5 males at the dose of 62.5 mg/kg.

Hypertrophy of hepatocytes and increased mitosis of hepatocytes are considered as histopathological changes associated with the increased liver weight observed in this study. The liver hypertrophy is associated with an increase in absolute and relative weight of the liver in exposed animals. However, such changes do not meet the criterion of significant adverse effect as defined in section 3.9.2.7.3.e of CLP Regulation (multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity).

The focal necrosis incidence was found to be significantly increased in males, but not in female rats exposed to 62.5 mg/kg. The increased incidence of focal necrosis is in agreement with the dose-dependent and significantly increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and with changes in other biochemical parameters occurring in rats exposed to 62.5 mg/kg. This is considered as sufficient evidence that UV-320 induced significant, adverse changes which meet the classification criteria for STOT RE.

In the 28-day study of Hirata-Koizumi *et al.* (2008a) castrated females and male rats were administered UV-320 (2-(2'-hydroxy-3',5'-di-tert-butylphenyl) benzotriazole) by gavage at doses of 0 (vehicle: corn oil), 0.5, 2.5 or 12.5 mg/kg bw/day for 28 days. The aim of this study was to explain the differences in sensitivity of male and female rats to the toxic properties of UV-320 as observed in a previous study of Hirata-Koizumi *et al.* (2007).

No deaths, clinical signs of toxicity, or changes in body weight or food consumption were found at any doses.

Blood biochemical examination revealed significant dose-dependent increases:

- albumin level increased significantly from 4.43 g/dL in control males to 5.03 mg/dL in male rats exposed to 12.5 mg/kg. A significant increase of albumin level (5.14 g/dL) was also noted in females exposed to 12.5 mg/kg in comparison to the controls (4.19 g/dL)
- levels of glucose increased from 176 mg/dL in control males to 199 and 196 mg/dL in male rats exposed to 0.5 mg/kg and 12.5 mg/kg, respectively, no increase in glucose level was noted in females rats,
- urea nitrogen (BUN) level increased significantly in males exposed to 12.5 mg/kg –from 15.8 mg/L to 19.7 mg/L, and in females from 20.0 mg/L to 23.2 mg/L .
- AST level increased significantly in males exposed to 12.5 mg/kg –from 61.1 U/L to 91.4 IU/L, and from 54.8 IU/L in control females to 62.4 IU/L in females exposed to 0.5 mg/kg, no increase in AST level was noted in females exposed to 2.5 and 12.5 mg/kg
- ALT level increased significantly in male rats exposed to 12.5 mg/kg –from 40.2 U/L to 55.5 U/L and no increase was noted in females exposed at any level



- ALP level increased significantly in male rats exposed to 12.5 mg/kg –from 868 IU/L to 1552.5 IU/L, and in female rats exposed to 12.5 mg/kg –from 727 IU/L to 1026 IU/L
- LDH level increased significantly in male rats exposed to 2.5 and 12.5 mg/kg –from 112 IU/L to 173 and 403 IU/L respectively , and in female rats exposed to 0.5, 2,5 and 12.5 mg/kg –from 138 IU/L to 254, 209 and 235 IU/L respectively
- creatinine level slightly decreased (to ca. 84% of the control value) in all exposed males, and females exposed to 2.5 and 12.5 mg/kg.

At necropsy, absolute liver weight was significantly increased in males from 15.5 g in the control group to 18.2 g, 21.6 g and 26.9 g in males exposed to 0.5, 2.5 and 12.5 mg/kg respectively. In females there was a significant increase in absolute liver weight from 14.5g in the control group to 27.0 g in females exposed to 12.5 mg/kg.

The histopathological examination was carried out on the organs of 10 animals per sex in each group and resulted in the following:

- very slight diffuse hypertrophy of hepatocytes was observed in 4, 10 and 10 out of 10 male rats examined which had been exposed to 0.5, 2.5 and 12.5 mg/kg/day, as well as in 2 and 9 out of 10 female rats exposed to 2.5 and 12.5 mg/kg/day. The cytoplasm of the hepatocytes was slightly eosinophilic.
- very slight to slight anisokaryosis was found in 1, 8 and 10 males exposed to 0.5, 2.5 and 12.5 mg/kg and in 5 and 8 females exposed to 2.5 and 12.5 mg/kg/day, which indicates disturbed production of erythrocytes.
- very slight to slight nucleolar enlargement in hepatocytes was found in 1, 10 and 10 males exposed to 0.5, 2.5 and 12.5 mg/kg and in 5 and 9 females exposed to 2.5 and 12.5 mg/kg/day
- very slight to slight decreased glycogen in hepatocytes was found in 1, 6 and 10 males exposed to 0.5, 2.5 and 12.5 mg/kg and in 2 and 8 females exposed to 2.5 and 12.5 mg/kg/day
- very slight increased mitosis of hepatocytes was found in 1 and 4 males exposed to 2.5 and 12.5 mg/kg and no change was seen in exposed females
- Very slight focal necrosis was observed in 3 out of 10 examined male rats exposed to 12.5 mg/kg, while in females, focal necrosis was observed in 3 and 2 out of 10 examined female rats exposed to 2.5 and 12.5 mg/kg

No substance-related histopathological findings were detected in the heart or the kidneys.

The nature of effects in the liver found in this study does not meet the criterion of significant adverse effect defined for specific target organ toxicity-repeated exposure and set out in section 3.9.2.7.3.c/e of CLP Regulation (consistent and significant adverse change in clinical biochemistry or multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity). Focal necrosis was noted only in 20 -30% of animals exposed to 2.5 and 12.5 mg/kg, and is consistent with liver cell damage in Hirata-Koizumi *et al.* 2007.

**A 52-week repeated dose toxicity study** with UV 320 was conducted according to OECD TG 452 under GLP (Hirata-Koizumi *et al.* 2008b). Twenty female and male rats per dose (CD(SD)IGS) were given UV 320 by gavage at 0, 0.1, 0.5, or 2.5 mg/kg/day (males) and 0, 0.5, 2.5, or 12.5 mg/kg/day (females). At the end of the 13-week administration period, 10 males and 10 females from

each group were euthanized for the assessment of hematology, blood biochemistry, organ weights, and macroscopic and microscopic findings. The remaining animals in all groups (10 rats/sex/dose) were fully examined at the completion of the 52-week administration period.

No substance-related deaths or clinical signs of toxicity were observed in any group; however, a lowered body weight was found from day 36 to the end of the 52-week administration period at a dose of 2.5 mg/kg in males.

Where urine analysis is concerned, after 13-week exposure, a significant increase in osmotic pressure and specific gravity was detected at 2.5 mg/kg in males. No changes were noted in other parameters of urinalysis in any UV 320-treated groups (numerical data not provided). Urinalysis after 52-week exposure revealed a significant increase in osmotic pressure at 0.5 mg/kg (and above) in males, while it was significantly decreased at 12.5 mg/kg in females. A significant increase in urine volume was also detected at 12.5 mg/kg in females (numerical data not provided).

#### Hematological examination after 13-week exposure:

##### Males

- hemoglobin level – dose dependent decrease in males exposed to 0.5 and 2.5 mg/kg (up to 92% of the control value at the highest dose)
- hematocrit – dose dependent decrease in males exposed to 0.5 and 2.5 mg/kg (up to 92% of the control value at the highest dose of 2.5 mg/kg)
- Red blood cell count was decreased to 94.3% of the control value in males exposed to 2.5 mg/kg
- Platelet count was increased to 126.2% of the control value in males exposed to 2.5 mg/kg.

##### Females

- hematocrit significantly decreased to 95.3% of the control value and mean corpuscular volume (MCV) decreased to 96.9% of the control value noted only at 12.5 mg/kg.

#### On hematological examination after 52-week exposure:

##### Males:

- hemoglobin level not significantly affected by the treatment
- red blood cell count significantly decreased to 90% and to 92.6% of the control value in the at 0.5 mg/kg and 2.5 mg/kg respectively
- hematocrit decreased to 92,1 % of the control values at 2.5 mg/kg in males
- platelet count was increased to 131.5% of the control value in the 2.5 mg/kg male group
- prothrombin time (PT) was significantly prolonged to 161.5% of the control value in males exposed to 2.5 mg/kg.

##### Females:

- platelet count was increased to 117.1% of the control value in females exposed to 12.5 mg/kg.

The RAC noted that hematological changes induced by UV 320 were of slight intensity and the degree of anemia does not meet the criteria (reduction in Hb at  $\geq 20\%$ ) defined in section 3.9.2.5.2 of the Guidance on the Application to CLP Criteria for STOT RE classification.

Blood biochemical examination after 13-week exposure:

Males:

- glucose serum level increased to 127% and 124% of the control value in males exposed to 0.5 and 2.5 mg/kg
- blood urea nitrogen increased in males exposed to 0.5 and 2.5 mg/kg (up to 123.3% of the control value at the highest dose), which indicates disturbance of kidney function
- alkaline phosphatase -ALP increased in males exposed to 0.5 and 2.5 mg/kg (up to 377.4% of the control value at the highest dose)
- increase in albumin/globulin ratio from 1.22 in controls to 1.67 and 2.09 in the 0.5 mg/kg and 2.5 mg/kg group respectively
- decrease in  $\alpha_2$ - and  $\beta$ -globulin from 7.1% and 15.2 % in controls to 5.9 % and 11.5 % in the 0.5 mg/kg and to 5.6 % and 9.9 % in the 2.5 mg/kg group respectively

Females:

- total protein increase to 108% of the control value in a dose of 12.5 mg/kg in females
- albumin increase to 107.6% of the control value at the highest dose
- $\alpha_2$ - and  $\beta$ -globulin decrease 5.6% and 12.6% in control females to 4.7% and 9.9% females exposed to 12.5 mg/kg control value in a dose of 12.5 mg/kg in females

There were no substance related changes in other blood biochemical parameters, including total bilirubin level.

Blood biochemical examination after 52 week exposure:

Males:

- alkaline phosphatase (ALP) significantly increased to 258% and to 400% of the control value in the 0.5 mg/kg and 2.5mg/kg group, respectively
- Blood urea nitrogen was increased to 140% of the control value only in males exposed to 2.5 mg/kg in males, which indicates disturbance of kidney function
- albumin, a dose dependent increase to 116.9% and to 127.2% of the control value in the 0.5 mg/kg and 2.5mg/kg group, respectively
- decrease in  $\alpha_1$ -globulin to 79.2% and 69.8% of control value and in  $\alpha_2$ -globulin to 81.3% and 66.7% of control value in the 0.5 mg/kg and 2.5mg/kg group
- decrease in  $\beta$ -globulin to 70.9% of the control value in 2.5 mg/kg male group
- albumin/globulin ratio was significantly increased to 140.5% and to 175% value in the 0.5 mg/kg and 2.5mg/kg male group, respectively.

Females:

- alkaline phosphatase (ALP) significantly increased to 150% of the control value in the 12.5 mg/kg female group
- Blood glucose level was increased to the 115.3% of the control value only in females at 12.5 mg/kg.

No substance-related changes were found in other blood biochemical parameters, including total bilirubin level (data not shown).

### Conclusion on clinical chemistry findings

In the opinion of the RAC the nature and intensity of the changes in biochemical parameters were similar in animals exposed to UV-320 for 90 days (13 weeks) and in animals exposed for 52 weeks. The fact that the moderate intensity of changes in clinical biochemistry observed after 13-week exposure was not enhanced after an additional 39 weeks of exposure suggests that these changes alone observed after 90-day exposure do not meet the criterion defined in the CLP Regulation as consistent and significant adverse change in clinical biochemistry.

### Pathological and histopathological examination after 13-week exposure

At necropsy after 13-week exposure, enlargement of the liver was observed in 5 out of 9 males at 2.5 mg/kg and in 1 out of 10 females at 12.5 mg/kg, and the absolute and relative liver weights were significantly increased at 0.5 mg/kg and higher in males, and at 12.5 mg/kg in females.

A significant increase in the relative weights of the brain, heart, kidneys, and testes were also found at 2.5 mg/kg in males after 13-week exposure, but the absolute weight was not significantly changed.

On histopathology, centrilobular hypertrophy of hepatocytes accompanied with eosinophilic granular cytoplasm was observed in the liver. The incidence of centrilobular hypertrophy of hepatocytes in males and females was significantly increased from 0 % in controls to 60% males in the 2.5 mg/kg group and from 0 % in controls to 60% of females at 12.5 mg/kg.

Focal necrosis was observed in 1 of control male, 1 male in the 0.5 mg/kg group and 2 out of 10 males in the 2.5 mg/kg group. In females focal necrosis was observed only in 1 out of 10 females in the 2.5 mg/kg group but in none of the females exposed to 12.5 mg/kg.

### Pathological and histopathological examination after 52-week exposure

At necropsy after 52-week exposure, enlarged liver was observed in 7 out of 10 males at 0.5 mg/kg, 9 out of 10 males at 2.5 mg/kg, and 5 of 9 females at 12.5 mg/kg. Light gray macules were grossly detected in the liver of 2 out of 10 males at 2.5 mg/kg and of 1 out of 9 females at 12.5 mg/kg.

Absolute and relative liver weights were significantly increased at 0.5 mg/kg and higher in males, and at 12.5 mg/kg in females. A significant increase in the relative weights of the brain, pituitary, thyroids, lungs, heart, kidneys, testes and epididymides at 2.5 mg/kg in males were also found, but no statistically significant change was noted in the absolute weight.

The increased incidence of centrilobular hypertrophy of hepatocytes accompanied with eosinophilic granular cytoplasm was observed in histopathological examination in none of the control animals, in 5 and 7 males out of 10 examined in the 0.5 mg/kg and 2.5mg/kg groups, respectively and in 4 females out of 9 examined in the 12.5 mg/kg group.

The lipofuscin deposition in hepatocytes, which indicate remnants of phagocytized cell debris was noted only in 6 out of 10 examined males exposed to 2.5 mg/kg, and in 2 out of 9 examined females exposed to 12.5 mg/kg.

The incidence of cystic degeneration of hepatocytes was noted in 2, 2 and 4 out of 10 examined males exposed to 0.1, 0.5 and 2.5 mg/kg, and in none of the control or exposed females.

The altered hepatocellular foci (clear cell foci) were found in the liver of 1, 7 and 6 rats out of 10 examined males exposed to 0.1, 0.5 and 2.5 mg/kg, respectively, and in none of the control or exposed females.

The incidence of focal necrosis was not statistically increased in any exposed group and focal necrosis was seen in 3 and 4 out of 10 examined male rats exposed to 0.5 and 2.5 mg/kg, respectively, and in 2 control female rats while it was not seen in exposed females.

#### Comparison of effects with classification criteria

It is concluded by the RAC that the most frequent histopathological change in animals exposed for 13 weeks to UV 320 was a centrilobular hypertrophy of hepatocytes having eosinophilic granular cytoplasm in the liver, which is not relevant for classification. The incidence of focal necrosis in the liver was not statistically increased in any experimental group after 13-week or 52-week exposure and this change was not considered also by the authors of the study as treatment related. There were indications on liver cell degeneration from 28-day, 13-week and 52-week studies at doses of 2.5 mg/kg and above. However, 100% incidence of this histopathological change in the 28-day study, was not observed in the 52-week study, where only 4 of 10 males showed cystic degeneration and lipofuscin deposition was found in the liver of 6 out of 10 males. Taking into account that a dose of 2.5 mg/kg is below the guidance value of 30 mg/kg for category STOT RE 1 (for 28 day study), these findings were not considered sufficiently robust to justify category 1 due to lack of detailed information on the severity of these lesions.

Degenerative/necrotic liver changes which are in compliance with the observed increased activity of AST occurred in all animals at exposure level of 62.5 mg/kg, which is above the guidance value for classification as STOT RE 1 (30 mg/kg/day for 28-day studies), but within the guidance values for classification to category STOT RE 2 (30 mg/kg <C ≤ 300 mg/kg). This high dose was not tested in 13-week and 52-week studies. The myocardial degeneration observed at 12.5mg/kg and above in 28-day study was considered as adverse health effect and support the proposed classification.

In conclusion, the RAC is of the opinion that the information provided shows that the substance UV-320 meets the criteria for classification in **STOT RE 2** with hazard statement **H373** "May cause damage to liver through prolonged or repeated exposure". The organ affected after repeated administration of UV-320 is the **liver**.

## **2-(2H-BENZOTRIAZOL-2-YL)-4,6-DITERTPENTYLPHENOL (UV-328)**

### **Summary of the Dossier submitter's proposal**

The dossier submitter (DS, German competent authority) proposed that UV-328 be classified as "specific target organ toxicity – repeat exposure" in sub-category 2 (STOT RE 2) and therefore be considered as toxic, complying with the 'T' criterion for PBT definition under the REACH Regulation. UV-328 is registered under REACH (REACH registration dossier for UV-328, 2013). The REACH dossier is available on the ECHA dissemination database<sup>1</sup> and has been taken into account for this opinion.

The basis for the STOT RE 2 classification is derived from a sub-acute (49-day)/sub-chronic (90-day) repeated dose toxicity study conducted in rats (TNO, 1968, reported by EPA<sup>2</sup>). Repeated oral (gavage) administration of UV-328 caused toxicity in several organs, in particular in the liver. Briefly, microscopic examination revealed occasional foci of necrosis and a slight proliferation of bile duct epithelia. Parenchymal cells were enlarged. In the kidney, tubular necrosis was observed in some males from the higher feeding levels.

The DS did not present detailed information, concluding that the liver, bile duct and kidney effects meet the classification criteria for STOT RE 2. In addition, the majority of registrants self-classify UV-328 as STOT RE 2. In conclusion, based on the provisions of Annex XIII, section 1.1.3 (c) of the REACH Regulation, the DS also concluded that UV-328 meets the 'T' criterion.

### **Comments received during public consultation**

Some comments provided support to the DS proposal on the identification of the substance as a SVHC. One MSCA which did not specifically address "specific target organ toxicity – repeat exposure" (STOT RE), emphasised the lack of robust information to conclude on the vPvB/PBT status. On the hazard profile of the phenolic benzotriazoles in general, a stakeholder (STO) mentioned that the specific substitution pattern on the phenolic group influences their toxicity. As a consequence, a general read-across may not be appropriate for all endpoints.

Regarding STOT RE, another MSCA requested additional details to properly assess the classification proposal. In particular, they requested clarifications on whether the adverse toxicity effects reported were increased in a dose-related and statistically significant way (in particular for kidney tubular necrosis and focal or multifocal necrosis in the liver).

One STO commented on the weakness of the 'T' criterion (STOT RE 2, based on a NOAEL of 100 ppm, i.e. 22 mg/kg bw/d) although it concluded that the substance formally meets the criteria for 'T'. Another STO agreed with the DS proposal, referring to the Lead Registrant that has also concluded positively on the 'T' criterion.

A third MSCA supported the DS's proposal on STOT RE 2 on the basis of toxicity occurring in several target organs (liver, bile duct, kidney, blood) with a NOAEL set at 100 ppm (22 mg/kg/d).

### **Assessment and comparison with the classification criteria**

In the opinion of the RAC the information provided in the SVHC Annex XV dossier is not sufficient to develop an opinion of a similar robustness to a CLH opinion, because the severity of the effects was not described in sufficient details and the effects were not analysed taking into account the guidance values (in mg/kg bw/day) provided in tables 3.9.2 and 3.9.3 of Annex I to the CLP Regulation. The severity of the effects was also not compared with criteria of severity and

<sup>1</sup> <http://echa.europa.eu/information-on-chemicals/registered-substances>

<sup>2</sup> <http://ofmpub.epa.gov/opthpv/quicksearch.display?pChem=100705>

significance of effects provided in sections 3.9.2.7 - 3.9.2.8 of Annex I to the CLP Regulation. The information on exposure was limited to a feeding level expressed in ppm as content of UV-328 in animal feed.

Therefore the original study report of Til *et al.* (1968) entitled 'Short-term (49-day) and sub-chronic (90-day) toxicity studies with "RY 1137" in rats' was used to prepare a robust study summary in order to enable the independent assessment of the results and their comparison with the classification criteria.

In addition, the original study report of Geigy (1970) entitled 'Three months Toxicity Study. Tinuvin 328. Dietary administration - Beagle Dogs' was summarized in this opinion.

The results of all studies were compared with CLP classification criteria for repeated target organ toxicity (STOT RE) provided in section on UV-320.

#### Summary of target organ toxicity induced by UV-328 after repeated exposure

The DS proposed to classify UV-328 for repeated toxicity using the results of the 90-day study on rats (Til *et al.* (1968)) which are summarised below.

2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328) was given to female and male rats in the feed at concentration of 100, 200, 400, 800 and 1600 ppm for 90 days (Til *et al.* 1968). The dose levels for males during the first and last two weeks of study amounted approximately to (respectively): 0 mg/kg bw/day, 13.2 - 5.8 mg/kg bw/day, 22.2 - 11.5 mg/kg bw/day, 48.7 - 25.6 mg/kg bw/day, 98.7 - 52.7 mg/kg bw/day and 190.9 - 120.5 mg/kg bw/day. For female rats in the same periods of study the dose levels were: 0 mg/kg bw/day, 13.1 - 6.8 mg/kg bw/day, 24.6 - 13.1 mg/kg bw/ day, 46.9 - 26.7 mg/kg bw/day, 91.7 - 56.0 mg/kg bw/day and 189.8 - 118.0 mg/kg bw/day.

No treatment-related deaths occurred at any feeding level. Decreased body weight occurred in the highest feeding levels: 190.9 - 120.5 mg/kg bw/day in males and 189.8 - 118.0 mg/kg bw/day in females.

Hematological examinations at week 12 revealed a dose dependent decrease of hemoglobin content in blood starting in males from a feeding level of 22.2 - 11.5 mg/kg bw/ day and in females from a dose level of 91.7 - 56.0 mg/kg bw/day. The extent of reduction of hemoglobin in males exposed to the highest feeding level amounted to 12% of the control group value, while in females exposed to the highest feeding level amounted to 6 % of the hemoglobin level in the control group. The extent of reduction in the percentage of packed cell volume (haematocrit or erythrocyte volume fraction) was proportional to the reduction of hemoglobin content.

Glucose 6-phosphatase activity in pooled livers and kidneys of 5 males and 5 females per group was increased at all levels including the lowest level (100 ppm). The increase of specific glucose 6-phosphatase activity was not dose-dependent. No other biochemical examinations were done.

The relative weights (expressed in g per 100 g of body weight) of the liver, kidneys and thyroid were increased. Average relative liver weights were distinctly increased at all feeding levels in both sexes. The extent of the liver enlargement after 3 months was much less than that observed in rats of the same dose group after 4 weeks. Relative kidney weights were increased at the three highest dose levels in males as well as in females. Relative thyroid weights were higher than those of the controls in both sexes starting from the dose level of 200 ppm. Relative spleen weights of male rats were increased at the 800 and 1600 ppm level, and relative testis weights were increased at the three highest dose levels.

#### Pathological examinations

Liver

Gross pathologic examination after 13 weeks revealed distinct enlargement and greenish-drab discoloration of livers. In males, discoloration was observed at all dose levels, in females only at 800 and 1600 ppm (91.7 – 56.0 mg/kg bw/day and 189.8 – 118.0 mg/kg bw/day, respectively).

Microscopic examination of the livers revealed hepatic damage at all dose levels in males and females, which decreased in severity with decreasing dietary levels of the test substance. Foci of necrosis were occasionally present in males, and in smaller number in females, at the 800 and 1600 ppm feeding levels – respectively in males at dose levels of 98.7 – 52.7 mg/kg bw/day and 190.9 – 120.5 mg/kg bw/day, and in females of 91.7 – 56.0 mg/kg bw/day and 189.8 – 118.0 mg/kg bw/day.

At these feeding levels, extremely enlarged parenchymal cells with very homogeneous, strongly eosinophilic cytoplasm, often containing yellowish-green, non-birefringent pigment granules and also big eosinophilic, hyaline droplets were found, in males more frequently than in females. The nuclei were often considerably enlarged, showed varying amounts of chromatin and many big nucleoli. There were an increased number of binucleated hepatocytes, a few pyknotic nuclei and necrotic cells. There was also slight proliferation of bile ducts.

The hepatic damage described above occurred to a lesser extent also at the 400, 200 and 100 ppm level. Foci of necrosis no longer present in males at these lower dose levels were visible incidentally in females. Parenchymal cells were distinctly enlarged also at the lowest feeding level, with nuclei varying in size, shape and quantity of chromatin. Yellowish-green pigment and eosinophilic droplets occurred only in males; an increased number of binucleated hepatocytes and a few necrotic hepatocytes were visible in both sexes. Slight proliferation of bile duct epithelium was found in some instances.

#### Kidneys

Greenish discoloration of kidneys occurred in males and females at the two highest feeding levels (800 and 1600 ppm).

Microscopic examination of kidneys revealed tubular nephrosis at the two highest feeding levels in males – respectively: 98.7 – 52.7 mg/kg bw/day and 190.9 – 120.5 mg/kg bw/day. This renal injury consisted of tubules with narrowed lumens and thickened basement membranes lined by epithelium with little and scarcely staining cytoplasm and big vesicular nuclei.

In females yellowish-brown pigment granules in the cytoplasm of proximal tubular cells were noticed at 200 ppm (24.6 – 13.1 mg/kg bw/ day) and above; the amount of the pigment increased with increasing levels of UV-328.

The RAC noted that the histopathological changes observed in the liver and kidneys in males rats exposed to 98.7 – 52.7 mg/kg bw/day meet the criteria of severe, adverse health effects defined for classification as STOT RE set in section 3.9.2.7.3. of Annex I to the CLP Regulation:

*"(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;*

*(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;*

*(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver);"*

In the study reported by Geigy (1970) beagle dogs received the test material 2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol (TINUVIN 328) for 3 months in their diet. The test material concentration in the diet was chosen and adapted in such a way that the following daily doses resulted in the five groups treated with



test material: 15, 30, 60, 120 and 240 mg/kg body weight. Each treated group consisted of 3 male and 3 female beagle dogs. In addition 5 male and 5 female beagle dogs served as control.

One male dog in the highest dose group died during the 8th week of test material administration. Depression of the food consumption and loss of body weight were observed in the higher dose groups. The dogs in which these symptoms were most pronounced showed a sleepy and weak behavior. The ophthalmic examinations did not reveal noteworthy findings.

#### Hematology

The test material produced a considerable number of toxic symptoms which were in general more pronounced in the male dogs than in the female dogs. It seems that some toxic effects in the male dogs were as strong as or even stronger in the 120 mg/kg-group than in the 240 mg/kg-group.

Changes in the erythrocytes and hemoglobin which consisted of a decrease in number of erythrocytes, diminution of packed cell volume, decrease in hemoglobin content of blood, increase of mean corpuscular volume and decrease of mean corpuscular hemoglobin concentration were seen in dogs treated with 120 mg/kg and 240 mg/kg.

The mean red blood cell counts in male control dogs and in male dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months amounted, respectively to  $6.76 \times 10^6$ ,  $6.85 \times 10^6$ ,  $6.85 \times 10^6$ ,  $6.39 \times 10^6$ ,  $4.55 \times 10^6$  and  $6.99 \times 10^6$  / $\mu$ l.

The mean red blood cell counts in female control dogs and in female dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months amounted, respectively to  $7.13 \times 10^6$ ,  $7.47 \times 10^6$ ,  $7.27 \times 10^6$ ,  $7.29 \times 10^6$ ,  $6.84 \times 10^6$  and  $5.98 \times 10^6$  / $\mu$ l.

The mean hemoglobin concentration in male control dogs and in male dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months amounted, respectively to 15.7, 16.1, 15.8, 15.5, 10.5 and 16.3 g/100ml.

The mean hemoglobin concentration in female control dogs and in female dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months amounted, respectively to 16.2, 17.5, 17.2, 16.8, 16.2 and 13.1 g/100ml.

It is noted that reduction in level of hemoglobin in males at dose of 120 mg/kg, but not at dose of 240 mg/kg, and in females only in dose of 240 mg/kg were of  $\geq 20\%$  of hemoglobin level in the control animals thus meeting criteria of reduction in hemoglobin concentration as defined in section 3.9.2.5.2 of the Guidance in the Application to CLP Criteria for STOT RE classification. However, this effect does not warrant UV-328 to be classified as hematotoxic substance to STOT RE 2 category because the effect was observed only at doses above a guidance value of 100 mg/kg, and it was also not dose-dependent in males.

#### Clinical biochemistry

The glucose concentration in blood was affected by exposure in female and male dogs except for dog 240-5e exposed to 240/mg/kg, which died after 8 weeks of exposure.

The activity of GPT (glutamate pyruvate transaminase or alanine transaminase (ALT), GOT (glutamic-oxaloacetic transaminase or aspartate transaminase (AST) and alkaline phosphatase (ALP) in serum was increased. These enzyme activities mostly increased with the time of test material administration and/or included lower dose groups during the test material administration.

In the male dogs increased activities of these three enzymes were already seen in the 15 mg/kg-group; in the female dogs high activities of the serum ALP were found in the 15 mg/kg-group:

- alanine aminotransferase (ALT or GPT) level increased from a value of 7.8 mU/ml in control male dogs to values 70.9, 52.3, 119.9, 98.9 and 85.4 mU/ml, respectively in male dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months
- alanine aminotransferase (ALT or GPT) level increased from a value of 10.6 mU/ml in control female dogs to values 13.1, 48.5, 28.9, 59.7 and 96.1 mU/ml, respectively in female dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months
- aspartate aminotransferase (AST or GPT) level increased from a value of 13.4 mU/ml in control male dogs to values 41.1, 22.4, 42.9, 56.7 and 30.8 mU/ml, respectively in male dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months
- aspartate aminotransferase (AST or GPT) level changed from a value of 16.8 mU/ml in control female dogs to values 13.1, 21.5, 19.6, 27.5 and 66.7 mU/ml, respectively in female dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months
- alkaline phosphatase (ALP) level changed from a value of 22 mU/ml in control male dogs to values 96, 253, 236, 290 and 245 mU/ml, respectively in male dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months
- alkaline phosphatase (ALP) level changed from a value of 39 mU/ml in control female dogs to values 84, 206, 373, 207 and 498 mU/ml, respectively in female dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months.

Total protein in serum was diminished in exposed male and female dogs with the lowest value in dogs exposed to 240 mg/kg bw/day for 3 months being 86% of the control group value in exposed males and 81.5% of the control group value in exposed females.

Changes in the protein pattern (electrophoresis) in serum were observed in the 30 mg/kg-group and in higher dose groups. Prothrombin time, blood clotting time, urea-nitrogen as well as sodium and potassium concentrations in serum were normal.

An increased bilirubin concentration was determined in male dogs of all dose groups; dog 240-5e showed an extremely high figure.

- total bilirubin level in serum increased from a value of 2.5 mg/l in control male dogs to values 7.7, 5.4, 5.4, 6.0 and 2.85 mg/l, respectively in male dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months; bilirubin level determined just before death in dog which died after 8 weeks of exposure was above 180 mg/kg.
- total bilirubin level in serum female dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months was not affected by the treatment.

#### Pathological examinations

At necropsy after 3 months of exposure 1 out of 3 male dogs exposed to 120 mg/kg and 1 male dog exposed to 240 mg/kg, which died after 8 weeks of exposure, had icterus universalis (jaundice). One out of 3 female dogs exposed to 240 mg/kg showed a slighter degree of icterus.

At necropsy, absolute liver weight was significantly increased in male dogs from 399g in control group to 583g, 494g, 565g, 408g and 436g in male dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months, respectively. In females there was a significant increase in absolute liver weight from 303g in control

group to 363g, 470g, 464g, 525g and 353g in female dogs exposed to 15, 30, 60, 120 and 240 mg/kg bw/day for 3 months, respectively.

The liver is severely affected by administration of UV-328. Several of the changes appeared already in the 15 mg/kg-group, e.g. fatty changes in Kupffer cells (specialized macrophages located in the liver lining the walls of the sinusoids), protein globules in cytoplasm, yellow pigmentation in Kupffer cells and Kupffer' cell hyperplasia; fatty degeneration of hepatocytes was only seen in dogs of 60 mg/kg-group and in higher dose groups.

The following changes were observed in the liver of male and female dogs:

- Kupffer cell hyperplasia: in 1 control female, in all 3 females exposed to 15 mg/kg, in one female and 1 male dog exposed to 30 mg/kg, in 2 males and 3 females exposed to 60 mg/kg; in 2 males and 2 females exposed to 120 mg/kg; in 3 males and 2 females exposed to 240 mg/kg;
- Fatty changes in Kupffer cells: in 1 male at 15mg/kg, 3 males at 30 mg/kg, and 1 female at 120 mg/kg
- Fatty degeneration of hepatocytes:
  - o monocellular fatty changes: in 3 males at 60 mg/kg, 3 females at 240 mg/kg
  - o focal fatty degeneration, partly central and partly peripheral: in 2 females at 60 mg/kg, 2 males at 120 mg/kg, and 2 males at 240 mg/kg
  - o diffuse fatty degeneration: in 1 male dog at 240 mg/kg
- Centrolobular cholestasis: in 1 male at 60 mg/kg, 120 mg/kg and 240 mg/kg
- Monocellular necrosis: in 1 male and in 1 female at 30 mg/kg and in 1 female at 240 mg/kg
- Fibrosis: in 1 male at 60 mg/kg
- Inflammation: in 1 male at 60 mg/kg and 1 male at 240 mg/kg.

According to the study, the findings did not indicate a clear relationship between dose and the strength of the changes of the liver.

#### Comparison of effects with classification criteria

Focal necrosis of the liver and tubular nephrosis caused by UV-328 in male and female rats (Til *et al.*, 1968) at the feeding level of 800 ppm (respectively in males at dose levels of 98.7 – 52.7 mg/kg bw/day, and in females of 91.7 – 56.0 mg/kg bw/day), meet the criteria of significant toxic effects, of relevance to human health, produced at exposure concentrations meeting guidance values for category STOT RE 2 ( $10 < C \leq 100$  mg/bw /day).

The pathological changes in the liver and kidneys observed at lower dose levels of 100 ppm (ca. 10mg/kg/day), 200 ppm (ca. 15 mg/kg/day) and 400 ppm (ca. 30 mg/kg bw/day) do not meet the criteria of severity and toxicological significance defined in CLP criteria such as multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity or severe morphological changes that are potentially reversible but provide clear evidence for marked organ dysfunction (e.g. severe fatty change in the liver).

Small changes in clinical biochemistry such as increased glucose 6-phosphatase activity in pooled livers and kidneys observed at the dose level of 100 ppm corresponding to ca. 10mg/kg bw/day or in hematology such as small reduction (less than 5% in hemoglobin concentration and in packed cell volumes) observed in males, but not in females at the dose level of 200 ppm corresponding to ca. 15

mg/kg bw/day are of minimal toxicological importance and does not warrant classification.

The histopathological effects observed in dogs exposed to 60 mg/kg such as fatty degeneration of hepatocytes and fibrosis meet the criteria of classification in STOT RE hazard class defined in section in 3.9.2.7.3.(d)–(g) of CLP Regulation.

The considerable changes in the activity of several enzymes in serum and changes observed in protein pattern in serum in animals exposed to 15 mg/kg or higher support classification as STOT RE. Taking into account that these effects were observed in exposure levels ranging from 10 to 100mg/kg bw/day, UV-328 should be classified in category 2 of specific target organ toxicity – repeated exposure.

In conclusion, the RAC is of the opinion that the information provided shows that the substance UV-328 meets the criteria for classification in **STOT RE 2** with the hazard statement **H373** "May cause damage to organs (**liver, kidneys**) through prolonged or repeated exposure".

## Supplemental information

Individual histopathological changes in reproductive organs of female and male dogs treated with UV-328 for 90 days (Geigy, 1970) are reported in the table below for information. Indeed, according to CLP Annex I, section 3.9.1.1, specific toxic effects covered by other hazard classes are not included in STOT-RE. STOT-RE should only be assigned where the observed toxicity is not covered more appropriately by another hazard class.

Table: Individual histopathological changes in reproductive organs of female and male dogs treated with UV-328 for 90 days (Geigy, 1970).

Dose in mg/kg bw/day	0 (control)	15	30	60	120	240
<b>Testis</b>						
	<b>Dog C-3</b> Tubules with only Sertoli cells		<b>Dog 30-3*</b> : several giant spermatogonia and multinucleated giant cells in tubules	<b>Dog 60-3</b> : several multinucleated giant cells in tubules and a slight chronic inflammation in the capsule  <b>Dog 60-5</b> : strong defect in spermiogenesis, a distinct atrophy of the tubules, hyperchromia and hyperplasia of the spermatogonia and multinucleated giant cells.	<b>Dog 120-1</b> : moderate atrophy  <b>Dog 120-3</b> : disturbances of spermiogenesis and progressing atrophy  <b>Dog 120-5</b> : disturbances of spermiogenesis, round cell infiltration of interstitial tissue with hyperplasia of Leydig' cells and nearly no spermatocytes in the lumina of tubules	<b>Dog 240-3</b> : disturbances of spermiogenesis, slight atrophy of tubular epithelium and multinucleated giant cells  <b>Dog 240-5e**</b> : disturbances of spermiogenesis, slight atrophy of tubular epithelium and multinucleated giant cells
<b>Prostate</b>						
			<b>Dog 30-5</b> : slight atrophy of the glands and a slight increase of stroma	<b>Dog 60-1</b> : glandular epithelium is slightly flattened  <b>Dog 60-5</b> : atrophy of the glands and a increase of stroma	<b>Dog 120-3</b> : slight atrophy of the glands  <b>Dog 120-5</b> : very strong atrophy and sclerosis of the stroma.	<b>Dog 240-3</b> : very strong atrophy and sclerosis of the stroma  <b>Dog 240-5e**</b> : very strong atrophy and sclerosis of the stroma
<b>Uterus</b>						
				<b>Dog 60-2</b> : slight atrophy of all layers of the uterus wall	<b>Dog 120-4</b> : slight atrophy of all layers of the uterus wall	<b>Dog 240-6</b> : slight atrophy of all layers of the uterus wall  <b>Dog 240-4</b> : atrophy of all layers of the uterus wall

\*First number is a dose level in mg/kg followed by a number of male (1,3 and 5) or female (2,4 and 60) dog in this study; \*\* dog 240-5e died after 8 weeks of treatment; dogs were mature at study start (male dogs: 35 weeks; female dogs 32 weeks)

No histopathological examinations of male and female reproductive organs of rats were done in the 90-day study of Til *et al.* (1968) study, but in male rats exposed to dose levels of 48.7 – 25.6 mg/kg bw/day, 98.7 – 52.7 mg/kg bw/day and 190.9 – 120.5 mg/kg bw/day the relative weight of testis was significantly increased.

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ANNEXES

- Annex 1 Request from the Executive Director of ECHA to the RAC of 4 April 2013 I(2013)0093 – ‘the mandate’.
- Annex 2 Annex XV dossier – Proposal for identification of a substance as a CMR 1A or 1B, PBT, vPvB or a substance of an equivalent level of concern (UV-320)
- Annex 3 Annex XV dossier – Proposal for identification of a substance as a CMR 1A or 1B, PBT, vPvB or a substance of an equivalent level of concern (UV-328)

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