COMPILED COMMENTS ON CLH CONSULTATION

Comments provided during consultation are made available in the table below as submitted through the web form. Please note that the comments displayed below may have been accompanied by attachments which are listed in this table and included in a zip file if non-confidential. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Last data extracted on 15.11.2023

Substance name: fluazaindolizine (ISO); 8-chloro-*N*-[(2-chloro-5-methoxyphenyl)sulfonyl]-6-(trifluoromethyl)imidazo[1,2-a]pyridine-2-carboxamide
CAS number: 1254304-22-7
EC number: -
Dossier submitter: Malta

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	1
Comment received				
A parallel peer review process for active substance approval according to Reg. (EC) No 1107/2009 is ongoing. Phys Chem: In Table 1 of section 2.2.1 of the CLH report, the information given under endpoint “Relative density” seems to be misplaced. Also, in the table no information regarding water solubility is given, despite being addressed in the text above the table.				

PHYSICAL HAZARDS

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	2
Comment received				
Has not been reviewed by DK.				

HEALTH HAZARDS – Acute toxicity

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	3
Comment received				
Based on the available data, we agree that classification is required for acute oral toxicity, Acute Tox. 4, H302. We also agree with the proposal that classification for dermal and inhalation toxicity is not required for fluazaindolizine.				

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	4
Comment received				
Acute oral: DK agree with the overall conclusion to classify as Acute tox class. 4. However, based on the results in study KCA 5.2.1/02 (1/3 animals died at 940 mg/kg bw, 2/2 animals died at 1500 mg/kg bw), it is not quite logical how an LD50 of 940 mg/kg bw was derived. Acute dermal: DK agree with the overall conclusion. Study KCA 5.2.2/03 is non-GLP and an LD50 should, therefore, be derived with caution for this study.				

HEALTH HAZARDS – Skin corrosion / irritation

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	5
Comment received				
We agree with the proposal that classification for skin corrosion/irritation is not required for fluazaindolizine.				

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	6
Comment received				
Has not been reviewed by DK.				

HEALTH HAZARDS – Serious eye damage / eye irritation

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	7
Comment received				
We agree with the proposal that classification for serious eye damage/eye irritation is not required for fluazaindolizine.				

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	8
Comment received				
Has not been reviewed by DK.				

HEALTH HAZARDS – Respiratory sensitisation

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	9
Comment received				
There was no evidence of respiratory irritation in single-dose inhalation studies in rats. There is no reported evidence of respiratory sensitisation in humans.				

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	10

Comment received
Has not been reviewed by DK.

HEALTH HAZARDS – Skin sensitisation

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	11
Comment received				
Based on the weight-of-evidence assessment taking into account the available studies of skin sensitisation testing different batches of technical material, we agree that it is reasonable that the pure active substance fluazaindolizine can be considered not skin sensitising. No classification for skin sensitising properties is warranted for pure fluazaindolizine. It is reasonable to conclude that an impurity in batch DPX-Q8U80-181 was responsible for the positive results. Note: During the parallel peer review process for active substance approval according to Reg. (EC) No 1107/2009, we commented that the maximum content of impurity 2 (IN-QJA58) in the specification of the technical material has to be reduced to 1.4 g/kg; otherwise, the technical material has to be classified as Skin Sens. 1, H317. Justification: There are four reports examining fluazaindolizine skin sensitization, with different batches: negative (GPMT): Batch DPX-Q8U80-068 (99.6 % pure) positive (GPMT, LLNA): Batch DPX-Q8U80-181 (94.8 % pure) negative (LLNA): DPX-Q8U80-068 (99.6 % pure), DPX-Q8U80-093 (98.5 % pure), DPX-Q8U80-200 (96.9 % pure), TSN315809 (98.1 % pure). According to the available data (GPMT, LLNA), only the least pure batch Q8U80-181 (94.8 %) is skin sensitising. This batch contains impurity 2. Only one other batch containing this impurity is tested for skin sensitising properties (TSN 315809) and was not skin sensitising but the level of purity is higher in this batch (98.1 %), and the level of impurity is lower. We have also noted that it has not been fully clarified due to which impurity (and which concentration thereof) batch Q8U80-181 gave repeated positive results in skin sensitising studies. In any case, for classification purposes of the pure active substance, studies conducted with higher purity batches (see above, 99.6 %, 98.5 %, 96.9 %, 98.1 %) should be given more weight.				

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	12
Comment received				
Skin sensitisation: DK agree with the outcome of the WoE assessment that no classification for skin sensitisation is warranted.				

HEALTH HAZARDS – Germ cell mutagenicity

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	13
Comment received				
Based on an overall conclusion considering the available data, we agree that classification is				

not required for mutagenicity.

Fluazaindolizine was extensively tested in a battery of appropriate tests. Five reverse mutation assays in bacteria were available in which different batches were tested in the same four strains of Salmonella typhimurium and one Escherichia coli strain, all with negative results; however, some studies should be considered supplementary only due to missing confirmatory experiments. Two in vitro tests on gene mutation in mammalian cells also gave negative results. However, in two in vitro chromosome aberration assays, fluazaindolizine was tested positive for clastogenicity with and without metabolic activation. Four in vivo studies (three micronucleus assays in the mouse and another micronucleus assay in rats, and one UDS test in the rat) were conducted due to the positive in vitro results. While two micronucleus assays in the mouse are clearly negative, one should be considered equivocal due to statistically significant increases in MPCE but without clear dose-response relationship, in contrast to the assessment of the DS. In addition, the available micronucleus assay in rats should be considered inconclusive. It also should be noted that the available in vivo UDS test in rats should be considered supplementary only due to unknown sensitivity. According to the EFSA Journal 2017;15(12):5113, the use of the UDS is no longer recommended as a follow-up of positive in vitro test. Test results may be considered adequate for assessing genotoxic potential only in cases of positive results in this test. Due to the low sensitivity of the UDS in detecting in vivo genotoxicants, the result of the UDS is of lower predictive value and is no longer regarded suitable as a follow-up of positive in vitro tests.

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	14
Comment received				
Structural aberrations are observed in vitro both with and without metabolic activation. However, several in vivo studies are negative for this effect. Bone marrow exposure was demonstrated in one of the in vivo studies. DK agree with the overall conclusion that Fluazaindolizine is not genotoxic.				

HEALTH HAZARDS – Carcinogenicity

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	15
Comment received				
Based on the currently provided information from the available data, we agree that classification for carcinogenicity is not required for fluazaindolizine. However, further information should be amended by the DS to enable a substantiated conclusion on this endpoint: Long-term/carcinogenicity study in rats An adequate statistical analysis of neoplastic lesions as well as primary tumours taking into account the high mortality rate should be conducted to enable study evaluation. Justification: Obviously, no statistical analysis of tumour development is provided. Further, especially for neoplastic lesions, the statistical analysis (Cochran-Armitage test for trend) did not take into account survival, although survival was < 50 % in all groups. Statistics should include adjustment for survival, analysis of cumulative tumour risks relative to survival duration and analysis of the time to tumour. A comparison of incidences in different				

groups (survivors, decedents, total animals) and e.g., survival adjusted tests (e.g. poly-k tests) should be provided to enable evaluation of the study. In females, a statistically significant decrease in the survival rate was observed in the top dose compared to control. A simple statistical analysis which does not account for inter-current mortality can underestimate the carcinogenic effects if the treatment decreases survival.

In addition, incidences of non-neoplastic findings in the rat after 2 years of administration as provided in Table 263 should be further elaborated. For all dose groups, the number of animals/group is 70, although survival was < 50 % in all groups. Were all animals examined? Is it required to account for time to death? E.g. could the findings of kidney hyperplasia, transitional cell (males, n = 7-5-6-12-13) and hyperplasia, urothelial cell (males, n = 4-6-5-9-22), be relevant effects already at 1500 ppm?

Long-term/carcinogenicity study in mice
Reporting of the carcinogenicity study in mice is insufficient. The DS should provide tabulated results on mortality, organ weights, tumour incidences, non-neoplastic lesions, neoplastic lesions to enable study evaluation and a substantiated experts' discussion.

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	16
Comment received				
Has not been reviewed by DK.				

HEALTH HAZARDS – Reproductive toxicity

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	17
Comment received				

Adverse effects on sexual function and fertility
We agree with the DS that based on the available data from a 2-generation study as well as a one-generation study in rats, classification of fluazaindolizine as toxic for sexual function and fertility is not warranted.

Adverse effects on development:
We agree with the DS that based on the available data from developmental studies in rats as well as in rabbits, the effects are not sufficient for classification of fluazaindolizine as suspected of damaging the unborn child.

In the developmental rat study (2017), a minimal, but statistically significant increase in the incidence of the common foetal variation, short cervical ribs, was observed. In contrast to the DS, the observed increase in short cervical ribs at the top dose should be considered dose-related: The fetal incidence of this finding was still within the historical control range, although in nine fetuses it reached the upper edge of what had been seen in only one study from the HCD database. The more important parameter, litter incidence, exceeded the historical control range. A zero incidence, as observed in the concurrent control group, is a common finding and must not be used to disregard the high-dose observations. In the presence of maternal toxicity, this finding is sometimes observed. However, in three of the dams with litters in which the variation was found, terminal body weight was very close to the group mean of 365.6 g and in a fourth case was even much higher (420.1 g). Accordingly, there is no obvious relationship between occurrence of short cervical rib and maternal toxicity when individual data is taken into account. However, overall, this minor

skeletal variation is usually completely reversible and not regarded as relevant for classification.

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	18
Comment received				
Has not been reviewed by DK.				

HEALTH HAZARDS – Specific target organ toxicity - single exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	19
Comment received				
We agree with the proposal that specific target organ toxicity after single exposure relevant for classification of fluazaindolizine was not observed.				

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	20
Comment received				
Has not been reviewed by DK.				

HEALTH HAZARDS – Specific target organ toxicity - repeated exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	21
Comment received				
We support the DS proposal for classification with STOT RE 2, H373 (liver). We consider the observed liver effects sufficiently significant to warrant this classification, although some uncertainties should be pointed out in more detail. Justification: In the 28-d dog study, clinical chemistry was correlated with single cell necrosis at 138.7 mg/kg bw/d (< 300 mg/kg bw/d). This study should be considered supplementary only (2 animals / dose and sex only), however, effects should be considered sufficiently significant to indicate liver toxicity. In the 90-d dog study, single cell necrosis is observed from 1500 ppm (58.6 mg/kg bw/d). At the top dose of 4000 ppm (68.2 mg/kg bw/d for males, 92.7 mg/kg bw/d for females), single cell necrosis was observed in all animals in both sexes in the liver (< 100 mg/kg bw/d). In contrast to these findings below the threshold for classification, in the 1-yr dog study, significant liver toxicity findings were observed above the classification threshold for STOT RE 2: at 1000 ppm (35.8 mg/kg bw/d), clinical findings (ALP levels) were clearly adverse and statistically significant throughout the study duration (day 90: +380 %, day 181: +326 %, day 363: +310 %), but without clear histopathological correlates. Liver weight increases were not statistically significant, however up to 15-20 % (abs. and rel.). At the next higher dose (2000 ppm = 66.4 mg/kg bw/d), histopathological correlated findings were reported in females (2/4 females showed single cell necrosis), ALP levels were increased up to +717 % in males, and rel. liver weight increases were stat. sign. (35 %).				

However, it should be taken into account that additionally, one female died with reported marked liver toxicity at the top dose of 66.4 mg/kg bw/d.

Overall, we consider the classification with STOT RE 2, H373 (liver) to be justified.

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	22
Comment received				
Guidance values from CLP should not be interpreted strictly as stated in Guidance on the Application of the CLP Criteria, 2017, page 463. It is therefore not a valid stand-alone argument for not classifying based on urinary tract effects in rodents. In the WoE assessment of STOT-RE 2, considerations regarding consistency across studies of effects concerning the urinary tract should be included. DK agree that effects on the liver is relevant for classification as STOT-RE 2 considering the consistency across studies in dogs. Effects does not seem to be due to a general adaptive response following chemical exposure.				

ENVIRONMENTAL HAZARDS – Hazardous to the aquatic environment

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2023	Denmark		MemberState	23
Comment received				
Has not been reviewed by DK.				

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	United Kingdom	Health and Safety Executive	National Authority	24
Comment received				
Fluazaindolizine (ISO) (EC-; CAS: 1254304-22-7) We are unclear if Aquatic Chronic 2 is applicable given the comments below on the long-term toxicity endpoints for invertebrates and fish: In the CLH report, the key long-term endpoint is a 28-d EC10(repro) of 0.31 mg/L for the mysid Americamysis bahia. This endpoint is based on a steep dose-response and is around a factor of 4 below the statistical NOEC(repro) of 1.2 mg/L (mean measured concentrations) which exhibited around 2% reduction in the number of live offspring – the next treatment (LOEC) exhibited ~60% reduction. Reflecting the statistical uncertainty from this steep dose-response, the reproduction EC10 95% confidence interval is from 'not determined to 0.72 mg/L'. In this instance, we therefore wonder whether the NOEC(repro) is more robust for hazard classification. This NOEC is in the same range as the EC10(female weight) of 1.2 mg/L (mean measured concentrations) based on a clear dose-response above the NOEC of 0.62 mg/L for this endpoint. Considering ECHA, 2017, the reliable EC10(female weight) is preferred to the NOEC for this endpoint. Overall, the more robust NOEC(repro) and EC10(female weight) values indicate hazard classification endpoints in the range 1-10 mg/L. Noting the uncertainty regarding the key CLH endpoint, there appears to be similar uncertainty regarding wider long-term endpoints:				

In an OECD TG211 study with Daphnia magna, significant adult mortality was observed which potentially impacts the statistical analysis. While the RAR quotes a study NOEC of 0.63 mg/L (verified nominal concentrations) or 0.57 mg/L based on mean measured concentrations, this endpoint does not have a statistical basis as the study statistical NOEC was 1.3 mg/L (nominal - verified) / 1.2 mg/L (mean measured concentrations). Two EC10 endpoints are available for growth (adult dry weight) and reproduction (live neonates per surviving adults) – 0.51 mg/L and 1.5 mg/L (based on mean measured concentrations) respectively. However, the dry weight endpoint of 0.51 mg/L is below the statistical NOEC and exhibits 95% confidence intervals of <0.57 to 4.3mg/L. Noting the adult mortality in the study was greater than 20%, it is unclear if the statistical power was sufficient to consider the endpoints robust. Is there further information to address this point?

In an early life stage fish toxicity study with sheepshead minnow (Cyprinodon variegatus) dose-responses were observed to include positive impacts (particularly for length and growth) compared to pooled controls and a non-linear dose-response meaning there is some uncertainty with the statistical endpoints. However, EC10 values for relevant endpoints (dry weight and wet weight) are above the NOEC at 2.1 mg/L (95% CI <0.75-10 mg/L) and 5.5 mg/L (95% CI 2-12 mg/L) respectively. A second early life-stage toxicity test with rainbow trout (Oncorhynchus mykiss) resulted in no statistically significant effects and an unbounded NOEC reflecting the highest treatment of 12 mg/L (based on mean measured concentrations).

Noting the limitations and uncertainty regarding these endpoints, they do appear to present a commonality with more reliable endpoints in the 1-10 mg/L hazard classification range.

Reference: ECHA (2017) Guidance on the Application of the CLP Criteria.

Date	Country	Organisation	Type of Organisation	Comment number
09.11.2023	Germany		MemberState	25

Comment received

We agree to the proposed classification for environmental hazards.
We agree that fluazaindolizine is not readily biodegradable (page 241). But we miss the conclusion that the degradation in water is not rapid.
Further, we agree that there was no meaningful 14CO2 generated in either of the water systems in the OECD 309.

But, we do not agree that degradations in the water sediment systems in dark is also rapid (page 243). Further, we miss the unit “days” for of the given total system half-life in the two sediment systems from 20.6 to 51.4 under aerobic conditions. Based on this DT50 values the degradation is also not rapid.