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Letter

Derivation of permitted daily exposure value for 1,8-diazabicyclo[5.4.0]undec-7-ene in pharmaceutical products

Haruna Tahara[1], Hisakazu Sanada[2], Masayuki Mishima[2], Yasuyuki Ohnishi[3], Yu Haranosono[1] and Kiyohiro Hashimoto[4]

[1]Research & Development Division, Senju Pharmaceutical Co., Ltd., Hyogo, Japan
[2]Translational Research Division, Chugai Pharmaceutical Co., Ltd., Kanagawa, Japan
[3]Discovery Research Laboratory, Nippon Chemiphar Co., Ltd., Saitama, Japan
[4]Drug Safety Research Laboratory, Takeda Pharmaceutical Co., Ltd., Kanagawa, Japan

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ABSTRACT — The amidine compound 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is a strong organic base, is non-nucleophilic, and has been widely used in the organic synthesis of pharmaceuticals as a base catalyst. In the present study, we propose the permitted daily exposure (PDE) values of DBU by the oral, parenteral, and inhalation routes based on toxicological information in animals from the database of the European Chemicals Agency (ECHA). DBU exhibited mainly stomach toxicity caused by the corrosive effect of this strong alkali in rats. Although it is unlikely that stomach toxicity is induced in patients by DBU contamination of pharmaceuticals because most pharmaceutical products are adjusted to a near neutral pH, we calculated the PDE values conservatively based on the dosage at which no stomach-related findings were observed because no other noteworthy findings caused by systemic exposure were included in toxicity studies. By applying adjustment factors to the no-observed-adverse-effect-level (NOAEL) (120 mg/kg/day) in animal studies during the longest treatment period (3 months), we estimated the PDE values for oral, parenteral, and inhalation routes as 24, 12, and 6 mg/day, respectively. Derivation of the PDE was performed according to the concepts described by the International Conference on Harmonisation (ICH) Q3C(R8) and Q3D(R2) guidelines.

Key words: Permitted daily exposure (PDE), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), Impurity, ICH Q3C guideline, Pharmaceuticals

INTRODUCTION

The amidine compound 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is a non-nucleophilic strong organic base. DBU has been widely used for organic synthesis in the pharmaceutical industry as a base catalyst, complexing ligand, and non-nucleophilic base. The European Chemicals Agency (ECHA) regulates this compound under the Registration, Evaluation, Authorisation, and Restriction of Chemicals regulation (REACH) and 100−1000 tons of DBU per year is manufactured in and/or imported into the European Economic Area (ECHA, 2023).

A hazard assessment of DBU for workers and the general population was conducted for the inhalation and dermal routes according to the registration dossier infor-

Correspondence: Haruna Tahara (E-mail: haruna-tahara@senju.co.jp)

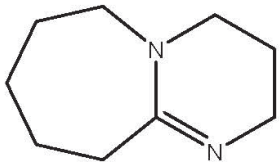

Fig. 1. 1,8-Diazabicyclo[5.4.0]undec-7-ene.

mation of the ECHA. The relevant starting point for the derivation of long-term systemic and dermal derived-no-effect-level (DNEL) values was the no-observed-adverse-effect-level (NOAEL) for oral toxicity of 120 mg/kg/day from a repeated oral dose toxicity study performed in accordance with the Organisation for Economic Co-operation and Development (OECD) test guideline 408. The long-term systemic inhalation and dermal DNEL values for workers are listed as 10.6 mg/m^3 and 3 mg/kg/day, respectively (ECHA, 2023).

To protect patients, determination of the permitted daily exposure (PDE) for the oral, parenteral, and inhalation routes could provide valuable information for the risk management of a control strategy for residual solvent in pharmaceutical ingredients. Here, we propose the PDE values for DBU based on the open access information of the ECHA website. Because many ECHA-registered studies were performed in accordance with OECD guidelines, we considered the ECHA database the most useful tool among the currently available sources of information on this compound compared with the other databases surveyed.

MATERIALS AND METHODS

Substance information

Identification

IUPAC name: 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine
EC name: 1,8-diazabicyclo[5.4.0]undec-7-ene
Trade name: DBU
CAS number: 6674-22-2
EC number: 229-713-7
Formula: $C_9H_{16}N_2$ (Fig. 1)
Molecular weight: 152.24

Chemical and Physical Properties

Appearance: Clear, colorless to pale yellow liquid
Solubility 24°C: Soluble in water, ethanol, and acetone
pH: 12.8 (10 g/L, H_2O, 20°C)
Log Pow (pH 7.0) < −2.2 at 25°C
Log Pow (pH 12.4) = −0.43 at 25°C
Log Pow (uncharged molecule) = 2.6968 at 25°C

Source of toxicological information

The existing information was searched in information sources including evaluation documents provided by international organizations, factsheets, databases, and studies. Derivation of the PDE was performed based on available information, mainly from the ECHA database (ECHA, 2023).

Information sources surveyed

1. Initial Risk Assessment Report/Chemical Substances Hazard Assessment Report, Chemicals Evaluation and Research Institute (CERI), Japan /National Institute of Technology and Evaluation (NITE) https://www.cerij.or.jp/evaluation_document/hazard_assessment_report_03.html
2. Japan Existing Chemical Data Base (JECDB), National Institute of Health Sciences (NIHS) https://dra4.nihs.go.jp/mhlw_data/jsp/SearchPage.jsp
3. Results of Carcinogenicity Studies Commissioned by Ministry of Health, Labor and Welfare (MHLW), Japan Bioassay Research Center (JBRC) https://anzeninfo.mhlw.go.jp/user/anzen/kag/carcino_report.htm
4. Environmental Risk Initial Assessment of Chemicals, Ministry of the Environment (MOE), Japan http://www.env.go.jp/chemi/risk/chemi_list/index.html
5. Recommendations for Working Environment Allowable Concentrations, Japan Society of Occupational Health (JSOH) https://www.sanei.or.jp/?mode=view&cid=309
6. OECD Screening Information Data Set (SIDS) report (SIDS Initial Assessment Report) https://www.echemportal.org/echemportal/substance-search
7. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, International Agency for Research on Cancer (IARC) http://www.inchem.org/pages/iarc.html
8. Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO) Joint Expert Committee on Food Additives – Monographs (JECFA Monographs) https://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx
9. Integrated Risk Information System (IRIS), United States Environmental Protection Agency (U.S. EPA) https://www.epa.gov/iris

10. National Toxicology Program (NTP) Database https://ntp.niehs.nih.gov/
11. NTP Report on Carcinogens (RoC) https://ntp.niehs.nih.gov/ntp/roc/content/listed_substances_508.pdf
12. NTP Technical Report https://ntp.niehs.nih.gov/data/tr/index.html
13. Toxicological Profile, Agency for Toxic Substances and Disease Registry (ATSDR) https://www.atsdr.cdc.gov/substances/index.asp
14. Priority Substances List Assessment Report, Canadian Environmental Protection Act (CEPA), Environment Canada, Health Canada https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/substances-list/priority-list.html
15. Pesticides "Reregistration Eligibility Decision", U.S. EPA https://archive.epa.gov/pesticides/reregistration/web/html/status.html
16. Test Data of Agricultural Chemicals, Food and Agricultural Materials Inspection Center (FAMIC) https://www.acis.famic.go.jp/syouroku/index.htm
17. Pesticide Safety Information, Japan Crop Protection Association https://www.jcpa.or.jp/labo/anzen/a.html
18. Risk Assessment Reports, Food Safety Commission of Japan https://www.fsc.go.jp/hyouka/
19. Study on the Review of Safety of Existing Food Additives, MHLW https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/shokuhin/syokuten/kizon/index.html
20. NITE Chemical Risk Information Platform (CHRIP) https://www.nite.go.jp/chem/chrip/chrip_search/systemTop
21. REACH Registered Substances Information, ECHA https://echa.europa.eu/
22. PubMed https://pubmed.ncbi.nlm.nih.gov/

Derivation of the PDE

The concept of PDE derivation described in the International Conference on Harmonisation (ICH) Q3C(R8) (ICH, 2021) and Q3D(R2) guidelines (ICH, 2022) was applied.

RESULTS AND DISCUSSION

Acute toxicity

DBU was administered orally by gavage to male and female Wistar rats (five animals/sex/group) at dosages of 0 (water), 215, 681, or 2000 mg/kg/day. Nine of 10 animals and all animals died within 1 day at dosages of 681 and 2000 mg/kg/day, respectively. Toxicity signs were observed, including a poor general state, dyspnea, apathy, abdominal position, staggering, tremor, twitching, cyanosis, exophthalmos, atonia, and paresis at these dosages. No abnormal findings were observed for a dosage of 215 mg/kg/day. At necropsy, general congestion was observed in some animals, and diapedetic hemorrhages in the stomach were observed in dead animals, while no obvious pathological findings were noted in surviving animals. The acute oral median lethal dose (LD_{50}) in rats was 215–681 mg/kg/day (ECHA, 2023).

Repeated dose toxicity

Repeated dose toxicity studies performed for 14 days, 29 to 57 days, and 3 months in rats were reported.

DBU was administered orally by gavage to male and female Crl:WI (Han) rats (three animals/sex/group) at dosages of 0 (water), 15, 40, 100, or 200 mg/kg/day once daily for 14 days. In the 200 mg/kg/day group, reddish foci on the stomach glandular mucosa were observed in all animals during the gross pathology evaluation. In addition, slight body weight loss or reduced body weight gain was noted, along with changes in clinical pathology parameters as a secondary effect of local gastric irritation. No toxicologically significant changes were observed in the other groups. The NOAEL was determined to be 100 mg/kg/day (ECHA, 2023).

Short-term (29 to 57 days) repeated dose toxicity was evaluated in combination with screening for reproductive/developmental toxicity. DBU was administered orally by gavage to male and female Crl:WI (Han) rats (five animals/sex/group) at dosages of 0 (water), 15, 50, or 150 mg/kg/day once daily. Males were exposed for 29 days, and females were exposed between 43 and 57 days (2 weeks before mating to day 4 of lactation). The absolute and relative kidney weights were dose-dependently higher for females in the treated groups than those in the control group. However, in the absence of any effects on relevant clinical pathology parameters or treatment-related findings noted in the kidneys during the microscopic examination, the increased weights were not considered to be biologically relevant. At a dosage of 150 mg/kg/day, parental effects on the stomach were observed in the macroscopic examination, including dark red foci and an irregular stomach surface. Corroborative findings, consisting of hemorrhage, degeneration of the glandular mucosa, inflammation, hyperplasia, and hyperkeratosis in the stomach were observed upon microscopic exami-

nation. All effects were local, limited to the stomach, and could be attributable to the strong alkaline corrosivity of the test substance. No toxicologically significant changes occurred in the other groups. The NOAEL was 50 mg/kg/day (ECHA, 2023). This study was performed in accordance with OECD test guideline 422 and Good Laboratory Practice (GLP) compliance.

DBU was administered orally by gavage to male and female Wistar rats (10 animals/sex/group) at dosages of 0 (water), 15, 40, or 120 mg/kg/day once daily for 3 months. At a dosage of 120 mg/kg/day, salivation was observed within 2 hr after treatment. On the basis of the temporary and short-term appearance immediately after dosing, we concluded that salivation was induced by the bad taste of the test substance or local effects on the upper digestive tract. No toxicologically significant changes were observed in any group. The NOAEL was 120 mg/kg/day (ECHA, 2023). This study was performed in accordance with OECD test guideline 408 and GLP compliance.

Genotoxicity

DBU was negative in the Ames test, *in vitro* micronucleus test with Chinese hamster lung fibroblast (V79) cells, gene mutation test with Chinese hamster ovary (CHO) cells, and *in vitro* comet assay with lymphocytes (ECHA, 2023). These studies, except for the *in vitro* comet assay, were performed in accordance with OECD test guidelines 471, 487, and 476, respectively, and GLP compliance.

Carcinogenicity

No relevant data were available.

Reproductive toxicity

Screening for reproductive/developmental toxicity was performed in combination with short-term repeated dose toxicity. DBU was administered orally by gavage to male and female Crl:WI (Han) rats (five animals/sex/group) at dosages of 0 (water), 15, 50, or 150 mg/kg/day once daily. Males were exposed for 29 days (2 weeks prior to mating, during mating, and up to termination), and females were exposed between 43 and 57 days (for 2 weeks prior to mating and during mating, gestation, and 4 days of lactation). For a dosage of 150 mg/kg/day, local effects on the stomach were observed in parent animals as described in the repeated toxicity test section. No reproductive or developmental toxicities were observed in treatment groups receiving up to 150 mg/kg/day. The NOAEL was estimated to be 50 mg/kg for parental toxicity and 150 mg/kg for reproductive and developmental toxicity (ECHA, 2023). This study was performed in accordance with OECD test guidelines 421 and 422 and GLP compliance.

To examine teratogenicity, DBU was administered orally by gavage to pregnant Wistar rats (25 animals/group) at dosages of 0 (water), 15, 50, or 150 mg/kg/day once daily from implantation to 1 day prior to the expected day of parturition (gestational day 6−19). No toxicologically significant changes were found during clinical observation, including food consumption or body weight changes in maternal animals in any group. In addition, there was no evidence for toxicologically relevant adverse effects of the test substance on fetal morphology at any dosage. The NOAEL was 150 mg/kg/day for both maternal and prenatal developmental toxicity (ECHA, 2023). This study was performed in accordance with OECD test guideline 414 and GLP compliance.

Skin/eye irritation

DBU was found to be corrosive in an *in vitro* skin corrosion test using a biobarrier membrane (ECHA, 2023).

Sensitization

No relevant data were available.

Safety pharmacology

No relevant data were available.

Bioavailability

Oral bioavailability was predicted for DBU according to Lipinski's rules, and the molecular weight, Log P value, and high water solubility favored oral absorption of DBU. Theoretical assessment indicated that the substance is hydrolytically stable; therefore, DBU is likely to be absorbed intact. In the aforementioned short-term (29 to 57 days) repeated dose toxicity study, an increase in kidney weight was observed, suggesting that a portion of DBU is absorbed and excreted in the urine. However, the study did not conclusively demonstrate the systemic exposure level. A default assumption of 50% oral absorption was made for risk assessment in the case that no experimental data were available (ECHA, 2012).

Other *in vitro* safety data

No relevant data were available.

Human exposure experiences

There were no data for human exposure.

The long-term systemic inhalation and dermal DNEL values for workers were calculated using the systemic NOAEL of 120 mg/kg/day from the 3-month repeat-

ed dose toxicity study. For the inhalation DNEL, the oral no-observed-adverse-effect concentration (NOAEC) was 105.8 mg/m^3, which was derived from a correction for the breathing rate (/0.38 × 0.67) and extent of absorption (× 50%/100%). Application of an overall adjustment factor of 10 (1 for the dose-response relationship; 2 for exposure duration; 1 for allometric factors; 1 for other interspecies differences; 5 for intraspecies differences; 1 for database quality; 1 for remaining uncertainties) resulted in a long-term systemic inhalation DNEL of 10.6 mg/m^3/day. No correction of the oral endpoint was performed for the dermal DNEL because oral and dermal absorption are considered to be comparable (worst-case assumption). Application of an overall adjustment factor of 40 (1 for the dose-response relationship; 2 for exposure duration; 4 for allometric factors; 1 for other interspecies differences; 5 for intraspecies differences; 1 for database quality; 1 for remaining uncertainties) resulted in a long-term systemic dermal DNEL of 3 mg/kg/day (ECHA, 2023).

Information from other sources

No relevant data were available.

Derivation of the PDE for the oral route

Stomach toxicity was the critical leading effect of DBU for the purpose of this assessment and was used as the basis for setting the PDE value. The findings observed in the stomach were considered to be caused by the corrosive effect of this strong alkali. Because most pharmaceutical products are adjusted to a near neutral pH, the alkaline property of DBU does not affect patients via contamination of pharmaceuticals. Therefore, we calculated the PDE conservatively based on the NOAEL for which no stomach toxicity was found.

Scenario 1: On the basis of repeated dose toxicity

The NOAEL was determined to be 120 mg/kg, for both males and females, for which no effects on the stomach were observed during the longest study (3 months).

PDE = 120 mg/kg/day × 50 kg/(F1 × F2 × F3 × F4 × F5) = 24 mg/day

Adjustment factors
F1 = 5 for extrapolation from rats to humans
F2 = 10 for variability among individuals
F3 = 5 for a study duration of 3 months in rats
F4 = 1 for the severity of systemic toxicity
F5 = 1 for the NOAEL; because only salivation, likely because of the bad taste of the test substance or local effects on the upper digestive tract, was observed at the NOAEL, we considered that the NOAEL was similar to the no-observed-effect-level (NOEL).

Scenario 2: On the basis of reproductive toxicity

The results of a reproductive toxicity study conducted by orally administering DBU to pregnant rats (in accordance with OECD test guideline 414, GLP-compliant) showed no developmental effects on the fetuses at dosages up to 150 mg/kg/day. The NOAEL was determined to be 150 mg/kg for the embryo-fetus.

PDE = 150 mg/kg/day × 50 kg/(F1 × F2 × F3 × F4 × F5) = 150 mg/day

Adjustment factors
F1 = 5 for extrapolation from rats to humans
F2 = 10 for variability among individuals
F3 = 1 for a reproductive study in which the whole period of organogenesis is covered
F4 = 1 for the severity of systemic toxicity
F5 = 1, we regarded the starting point as the NOEL

Judgment for the oral route

In consideration of safety, the lowest oral PDE, 24 mg /day, was proposed for DBU.

Derivation of the PDE for the parenteral route

Oral absorption of DBU is unclear. The parenteral PDE was calculated assuming that the oral absorption rate of DBU was 50%.

PDE parenteral = PDE oral × 50%/100%
= 24 mg/day × 50/100 = 12 mg /day

Derivation of the PDE for the inhalation route

According to the ECHA, the long-term systemic inhalation DNEL for workers is proposed to be 10.6 mg/m^3 (ECHA, 2023). However, the adjustment factor for calculating the inhalation DNEL for workers was different from that of the ICH Q3C(R8) and Q3D(R2) guidelines. Therefore, we calculated the inhalation PDE using the systemic NOAEL of 120 mg/kg/day from the OECD 408 study and taking into account the adjustment factor of the ICH Q3C(R8) and Q3D(R2) guidelines. The oral absorption of DBU was assumed to be 50%.

Inhalation NOAEC = PDE oral/daily respiration rate in rats × 50%/100%
= 120 mg/kg/day/(1.15 m^3/kg/day ×

1000 L/m^3) × 50%/100%
= 0.052 mg/L

Daily corrected dose = 0.052 mg/L × 28,800 L/day (daily respiration rate in humans)/50 kg = 30 mg/kg/day

PDE inhalation = 30 mg/kg/day × 50 kg/(F1 × F2 × F3 × F4 × F5) = 6 mg/day

Adjustment factors
F1 = 5 for extrapolation from rats to humans
F2 = 10 for variability among individuals
F3 = 5 for a study duration of 3 months in rats
F4 = 1 for the severity of systemic toxicity
F5 = 1 for the NOAEL; because only salivation caused by the bad taste of the test substance or local effects on the upper digestive tract were observed at the NOAEL, we considered that the NOAEL was similar to the NOEL.

Conclusion

By applying adjustment factors to the NOAEL (120 mg/kg/day) determined in the animal study with the longest treatment period (3 months), we estimated the PDE values for the oral, parenteral, and inhalation routes to be 24, 12, and 6 mg/day, respectively.

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Conflict of interest---- The authors are employees of pharmaceutical companies that intend to use DBU. Because the full report of each toxicology study was not disclosed, the authors considered it appropriate to use the NOAELs determined based on the open access information of the ECHA website. ECHA-registered studies were often performed by companies that had business interests in the test compound.

REFERENCES

ECHA. (2012): Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. Version, **2**, 1.
ECHA. (2023): information, EC number: 229-713-7. 1,8-diazabicyclo[5.4.0]undec-7-ene. https://echa.europa.eu/registration-dossier/-/registered-dossier/10251/7/1
ICH (2022): Guideline for elemental impurities Q3D(R2).
ICH (2021): Impurities: guidelines for residual solvents Q3C(R8).