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Letter

Derivation of the permitted daily exposure value for p-tert-butylphenol as an impurity in pharmaceutical products

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ABSTRACT — p-tert-butyl phenol (ptBP) is an alkylphenol organic compound that is used in the production of polycarbonates, phenolic resins, epoxy resins, etc., as a polymer chain terminator. However, it can occasionally be an impurity contaminant during the chemical synthesis of active pharmaceutical ingredients. Some alkylphenols are known as carcinogens or endocrine modulators, therefore controlling the impurity level to below the permitted daily exposure (PDE) value based on the toxicological risk assessment of ptBP should be considered. Here, we propose the PDE using toxicological information primarily obtained from OECD Existing Chemicals Database. Four scenarios for the oral PDE calculation were investigated, and the lowest value, 2 mg/day, was proposed as the most appropriate control criterion of ptBP based on the depigmentation toxicity seen in black mice. The PDE for the parenteral route was concluded to be 1 mg/day by applying a default bioavailability of 50% per ICH Q3D guideline.

Key words: PDE, p-tert-butylphenol, Impurity, ICH Q3C, ICH Q3D

INTRODUCTION

p-tert-butyl phenol (ptBP, Fig. 1) is an organic alkylphenol compound that is used in the production of polycarbonates, phenolic resins, epoxy resins etc., as a polymer chain terminator. It is included in the High Production Volume (HPV) Chemical List issued by the Organization for Economic Co-operation and Development (OECD). ptBP can occasionally be an impurity contaminant during the chemical synthesis of active pharmaceutical ingredients. Some alkylphenols, such as BHA (2(3)-tert-butyl-methoxyphenol, TBMP (2-tert-butyl-4-methylphenol), and BHT (2,6-bis-tert-butyl-methoxyphenol), are known as carcinogens, as well as p-nonylphenol and p-tert-octylphenol, which act as endocrine modulators. Therefore, the toxicological risk assessment of ptBP and determination of the permitted daily exposure (PDE) value should be considered.

The information on the HPV chemicals was obtained from the OECD Screening Information Data Sets (SIDS) created through the cooperative efforts of OECD countries. The collected ptBP toxicity data from the OECD test guidelines for chemicals (SIDS, 2000) were reliable and suitable for assessment. In addition, as further post-SIDS analysis, European Union Risk Assessment Report for ptBP was issued in 2008, and additional reliable data were available.

Since ptBP is currently not included in the International Conference on Harmonization (ICH) guidelines, we aimed to develop a ptBP risk assessment to calculate the PDE value based on available toxicological data in accordance with the ICH methodology (described in ICH Q3C and Q3D guidelines).

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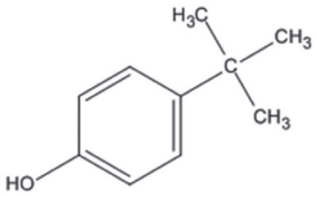

Fig. 1. Phenol, 4-(1,1-dimethylethyl).

MATERIALS AND METHODS

Substance information

Identification

IUPAC name: Phenol, 4-(1,1-dimethylethyl)
OECD name: p-tert-Butyl phenol
Synonyms: 4-tert-Butylphenol, 4-(t-butyl)phenol, Butylphen, 1-hydroxy-4-tert-butylbenzene, p-t-butylphenol
CAS number: 98-54-4
EC number: 202-679-0
Formula: $C_{10}H_{14}O$
Molecular weight: 150.22

Chemical and Physical Properties

Appearance: Crystals or practically white flakes
Solubility: 610 mg/L (water at 25°C), soluble in ethanol, ether, chloroform, alkalis
Melting point: 99.3°C
Log Pow: 3.29

Source of toxicological information

Many toxicological data of ptBP were obtained from the SIDS Initial Assessment Report (SIDS, 2000) for the 10th SIDS Initial Assessment Meeting (SIAM) and revised SIDS Initial Assessment Profile (2003), in which the *in vivo* genotoxicity study results were added. These data were presented at the SIAMs, and the assessment was finalized. Post-SIDS assessment was conducted in the context of the EU Existing Substance Regulation to investigate the potential for endocrine disruption and reproductive toxicity, and the results were issued in the EU Risk Assessment Report (2008). Additional and recent toxicological data were gathered from the following databases: GHS Classification Results by Japanese Government, National Institute of Technology and Evaluation (NITE)-Chemical Risk Information Platform (NITE-CHRIP, Japan), J-Check (MHLW, MOE, and NITE, Japan), Registry of Toxic Effects of Chemical Substances (RTECS), International Chemical Safety Cards (ICSC), JMPR Monographs, OECD Existing Chemicals Database, Hazardous Substances Data Bank (HSDB), US National Toxicology Program, Toxicological profiles of Agency for Toxic Substances and Disease Registry (ATSDR), Integrated Risk Information System (IRIS) Database for Risk Assessment for US EPA, PubChem and PubMed.

Toxicological data used for the risk assessment should derive from studies adopting adequately sound protocols such as those described in the OECD guidelines. In this work, reliable and recent toxicological data were extracted and used for the calculation of PDEs.

Derivation of PDE

The PDE derivation concept described in the ICH Q3C (ICH, 2021) and Q3D guidelines (ICH, 2020) was applied in this work.

RESULTS AND DISCUSSION

Acute toxicity

An acute oral study using five male and five female Sprague-Dawley (SD) rats receiving 2000 mg/kg of ptBP was conducted according to the OECD guideline 401 and GLP (EU Risk Assessment Report 2008). No deaths or signs of systemic toxicity were noted during the 14-day observation period. In a dose-range finding study in rats, males that were dosed at 5000 mg/kg died with clinical signs of hunched posture, lethargy, ptosis, red/brown stains around the snout and ataxia. No clinical sings were observed in females at 5000 mg/kg. These data indicated the low acute toxicity of ptBP when administered orally.

Repeated dose toxicity

A combined repeated oral dose toxicity study of ptBP was conducted using SD rats at doses of 0, 20, 60, and 200 mg/kg in compliance with the OECD guideline 422 and GLP. The administration period was 44 days in males and 46 days in females (14 days before mating to day 3 of lactation). No treatment-related changes were observed except for a noisy respiratory sound in females at 200 mg/kg. This symptom was due to the accidental aspiration of the irritant chemical at oral administration, and it was concluded that NOAEL for systemic toxicity was 200 mg/kg (SIDS, 2000). A different conclusion was indicated in the EU Risk Assessment Report (2008) based on the same study results. This report considered additional findings including a decrease in the mean plasma concentration of albumin in males at 60 and 200 mg/kg (6% and 13%) with a decrease in plasma protein in males at 200 mg/kg (6%), a decrease in erythrocyte count (5%), and an increase in white blood cell count (38%) in males at 200 mg/kg. Considering the respiratory distress in

females and several changes of blood parameters in males, the NOAEL was concluded to be 60 mg/kg in the EU Risk Assessment Report.

Genotoxicity

ptBP was negative in a bacterial reverse mutation assay using *S. Typhimurium* TA100, TA98, TA1535, and TA1537, and *E coli* WP2 *uvrA* with and without metabolic activation system (SIDS, 2000). In an *in vitro* chromosome aberration study, structural chromosome aberrations with metabolic activation and polyploidy with and without metabolic activation were induced in CHL/IU cells. However, high incidences of polyploidy were only observed at cytotoxic concentrations. This study was conducted according to the OECD guideline 473 and GLP.

A mammalian erythrocyte micronucleus study of ptBP was conducted using male CD-1 mice according to the OECD guideline 474 (SIDS Initial Assessment Profile, 2003). The animals received ptBP once intraperitoneally at doses of 0, 12.5, 25, and 50 mg/kg. No increase in the frequency of micronucleated bone marrow cells was noted at any of the dose levels at 24 and 48 hr after administration when compared to the control group. These results indicated that ptBP was not genotoxic *in vivo*.

Carcinogenicity

A two-stage promoting study was conducted using male Fisher rats at approximately 1.07 g/kg/day of ptBP for 51 weeks with or without N-methyl-nitrosoguanidine (MNNG) pre-treatment at 150 mg/kg (Hirose *et al*., 1988). The chemically induced forestomach squamous cell carcinoma was present in 75% of the animals after initiation with MNNG; however, no such carcinoma was found in animals treated with ptBP alone. The mechanism of forestomach tumor induction was likely a promoter effect. Meanwhile, forestomach hyperplasia was observed in the ptBP alone and ptBP with MNNG initiation groups, suggesting that ptBP has the potential to irritate the forestomach. The induction of forestomach tumors in rodents by agents without genotoxic potential may be of little relevance to humans (IARC, 2003). ptBP most likely does not have genotoxic potential as described above. Due to the lack of available carcinogenicity studies, the carcinogenic potency of ptBP was not sufficiently assessed; however, the EU Risk Assessment Report (2008) concluded that ptBP is unlikely to have carcinogenic potential.

Reproductive toxicity

Certain alkylphenols are recognized as endocrine modulators. The endocrine activity of ptBP was assessed through *in vitro* studies. There was a report that ptBP binds to the estrogen receptor with approximately 10,000-fold less affinity than 17-beta-estradiol, whereas it stimulated cell growth of the human breast cancer cell line MCF-7, and induced estrogen-regulated proteins, such as progesterone receptor (PgR) and estrogen-regulated secretarial protein (pS2) (Olsen *et al*., 2002).

As described above, a combined repeated oral dose toxicity study of ptBP was conducted using SD rats at doses of 0, 20, 60, and 200 mg/kg in compliance with the OECD guideline 422 and GLP (SIDS, 2000). In this study, there were no treatment-related toxic effects on pregnant and lactating females or their offspring, concluding that the NOAEL for the reproductive/developmental toxicity was 200 mg/kg.

A two-generation reproduction study of ptBP was conducted using SD rats with feeding administration at doses of 0, 800, 2500, and 7500 ppm (approximately 0, 70, 200, and 600 mg/kg/day, respectively) according to the OECD guideline 416, US EPA guideline OPPTS870.3800, and GLP (EU Risk Assessment Report, 2008). The results included: a reduction in body weight gain and food consumption during gestation and lactation; slight decrease in the number of implantation sites, live pups born, and viability of the pups; reduction in pup survival over days 1–4 of lactation; delay in vaginal opening and preputial separation in the F1 generation; increase in the incidence of primordial follicles with a concurrent decrease in the incidence of growing follicles at 7500 ppm; reduction in food consumption in the F0 and F1 generation prior to mating; decreases in pup body weights and litter weights with a smaller litter size in the F1 and F2 generation; marked increase in atrophy of the vaginal epithelium in the F0 and F1 generation; decrease in ovary weight in the F0 generation at 2500 ppm or more. No effects on mating performance, fertility, or gestation duration were reported. The NOAEL was concluded to be 800 ppm, which corresponds to 70 mg/kg/day in this study.

Skin/eye/respiratory irritation

A skin irritation study was conducted using three New Zealand rabbits according to the OECD guideline 404 and GLP (EU Risk Assessment Report, 2008). ptBP (500 mg) was moistened with distilled water and applied (semi-occluded) to intact skin for 4 hr. The results showed severe erythema and very slight to moderate edema was observed. Other adverse skin reactions included small areas of white-colored necrosis, well-defined erythema with scabs, thickening of the skin, and crust formation. These data indicated that ptBP irritated the skin although it was categorized as non-corrosive because there were no irreversible skin alterations according to the EC classifi-

cation criteria.

ptBP has been shown to cause severe irritation to the eyes although there were no studies performed in accordance with the OECD guidelines or GLP. An amount of about 80 mg of finely ground, dry powder ptBP was applied to the eyes of six New Zealand rabbits. The results showed severe corneal injury, iritis, and severe conjunctival irritation (Klonne *et al*., 1988). The corneal opacity was still significant 21 days after exposure, indicating that reversibility could not be established.

Respiratory irritation of ptBP was suggested in an inhalation study using SD rats. The animals were exposed for 4 hours to ptBP dust aerosol at 5600 mg/m^3 with the addition of vapor compound at 30 mg/m^3. The observed clinical sings included perinasal, perioral, and periocular mucosal irritation and respiratory distress (Klonne *et al*., 1988).

Sensitization

A recent skin sensitization study was conducted in accordance with the OECD guideline 406 and GLP (SIDS Initial Assessment Profile, 2003). The ptBP concentration was 0.5% in corn oil for the intracutaneous induction and 10% in Vaseline for the topical induction, with 1% in Vaseline for the topical challenge treatment. The challenge treatment caused no skin reaction, which suggested no evidence of skin sensitization.

Another study assessing the potential skin sensitization of ptBP and cross reactivity between ptBP and p-tert-butylcatechol (ptBC) was conducted according to the OECD guideline 406. The results showed one out of the 24 (4%) tested animals reacted positively. In addition, 9 out of the 24 (37.5%) animals subjected to induction with ptBC reacted positively to ptBP. These data suggested that ptBP have very low sensitization potential, and exposure of ptBC can lead to a cross-reaction with ptBP.

There were several patch studies in human for ptBP. However, these data had limited value because most of the studies showed very few positive results and were mainly performed on patients with former skin allergies or other skin diseases, or there was limited information about the exposure substance (EU Risk Assessment Report, 2008).

A clear conclusion had not been provided; however, the possibility of skin sensitization in human cannot be ruled out. It is thus classified as, “May cause an allergic skin reaction” according to EU CLP regulations.

Specific toxicity

There was a large amount of evidence for the depigmentation effect of ptBP. An oral administration study (3 times a week for 6 months) of ptBP was conducted using C57BL mice at 6 mg/day (EU Risk Assessment Report 2008). Diffuse or patchy depigmentation was observed in the majority of the animals, and the LOAEL for oral administration of ptBP was calculated to be 103 mg/kg/day (6 mg × 3 days divided by 7 days and divided by 0.025 kg body weight). Similar toxicity changes were reported from studies using colored mice, guinea pigs, or rabbits by subcutaneous injection, intramuscular injection, or cutaneous application, respectively. Histopathological examination of skin specimens with clinically observed depigmentation revealed the complete absence of melanin in a study using black guinea pigs (Gellin *et al*., 1979).

Depigmentation through occupational exposure to ptBP has been reported (EU Risk Assessment Report, 2008). In Germany, 23 workers handling ptBP showed depigmentation of the skin on their hands and arms, and some exhibited symmetrical depigmentation of body regions covered with clothing that could not be caused by direct exposure to the skin. It was likely caused by ingestion or inhalation. The depigmentation was reversible after several months when the material was removed from the skin.

Biochemical and cellular effects of ptBP on melanocytes have been reported. ptBP was oxidized by tyrosinase due to its structural similarity to the melanin precursor tyrosine, which results in the production of a reactive quinone, a strongly electrophilic substance, in melanocytes (Thörneby-Andersson *et al*., 2000). The reactive oxygen species damages these cells and induces apoptosis (Manga *et al*., 2006). *Ex vivo* immunological analysis in patients with vitiligo showed increased CD8$^+$ T cell reactivity against ptBP-exposed melanoma cells as well as moderate reactivity against unexposed cells. The autoimmune reactivity may mediate the systemic spread of vitiligo to unexposed body sites (Vrijman *et al*., 2019). In a murine model of atopic dermatitis (NC/Nga mice), the intraperitoneal administration of ptBP significantly exacerbated the skin lesions induced by mite allergy. It also tended to induce increases in the serum levels of IgE and antigen-specific IgG1, which suggested enhanced Th2-type immune responses (Sadakane *et al*., 2014). ptBP may have potential effects on the systemic immune system.

Safety pharmacology

No relevant data available

Bioavailability

No relevant data available

Other *in vitro* safety data

No relevant data available

Derivation of PDE for oral route

The PDE should be derived from the NOAEL in the most relevant animal study. For the present work, we proposed four scenarios and aimed to calculate the PDE for ptBP based on the data from the repeated dose toxicity, reproductive toxicity, carcinogenicity, and specific toxicity (depigmentation effect).

Scenario 1: Based on the repeated dose toxicity

There were two different NOAEL from the repeated oral dose toxicity studies at doses of 0, 20, 60, and 200 mg/kg in rats. One is 200 mg/kg based on no treatment-related changes in any dose levels, while the other is 60 mg/kg, which may be No-Observed-Effect-Level (NOEL), based on the respiratory distress in females and changes of several blood parameters in males at 200 mg/kg. Although it was difficult to judge whether either of conclusions was appropriate because raw data are not publicly available, the NOEL is recommended to calculate the PDE. Therefore, the lower NOAEL (60 mg/kg) was used conservatively for the PDE calculation.

PDE = 60 mg/kg/day × 50 (kg)/(F1 × F2 × F3 × F4 × F5) = 6 mg /day

Factor

F1 = 5 for extrapolation from rat to human

F2 = 10 for variability between individuals

F3 = 10 for study of a shorter duration (for 44 or 46 days of dosing)

F4 = 1 because no severe toxicity was encountered

F5 = 1 because the NOEL was determined

Scenario 2: Based on the reproductive toxicity

Two different types of studies, a combined repeated oral dose toxicity study and a two-generation reproduction rat study, were conducted to evaluate the reproductive toxicity of ptBP. In this work, the result of the two-generation reproduction study (NOAEL=70 mg/kg/day) was used for the PDE calculation because of the longer dosing period in the study.

PDE = 70 mg/kg/day × 50 (kg)/(F1 × F2 × F3 × F4 × F5) = 70 mg /day

Factor

F1 = 5 for extrapolation from rat to human

F2 = 10 for variability between individuals

F3 = 1 for reproductive study in which the whole period of organogenesis is covered

F4 = 1 because no severe toxicity was encountered

F5 = 1 because the NOAEL was determined

Scenario 3: Based on the carcinogenicity

A two-stage promoting rat study suggested that ptBP had promotor activity; however, the forestomach tumors in rodents were unlikely to be extrapolated to human (EU Risk Assessment Report 2008). In this work, ptBP was conservatively considered as a non-genotoxic carcinogen, and the PDE was calculated using LOEL in this study which was 1070 mg/kg/day.

PDE = 1070 mg/kg/day × 50 (kg)/(F1 × F2 × F3 × F4 × F5) = 10.7 mg /day

Factor

F1 = 5 for extrapolation from rat to human

F2 = 10 for variability between individuals

F3 = 1 for study that last at least one half-life time (1 year for rodent)

F4 = 10 for non-genotoxic carcinogenicity

F5 = 10 because only LOEL was available

Scenario 4: Based on the depigmentation effect

The involvement of occupational exposure and results of animal studies suggested that ptBP caused depigmentation in human. This effect is likely induced not only through direct contact with the skin but also through inhalation or ingestion routes. The result of a repeated oral dose study in black mice (LOAEL=103 mg/kg/day) was considered appropriate for the PDE calculation.

PDE = 103 mg/kg/day × 50 (kg)/(F1 × F2 × F3 × F4 × F5) = 2 mg/day

Factor

F1 = 12 for extrapolation from mice to human

F2 = 10 for variability between individuals

F3 = 2 for study of a shorter duration (for 6 months in rodents)

F4 = 1 because no severe toxicity was encountered

F5 = 10 because only LOAEL was available

Conclusion

To be conservative, in this investigation 2 mg/day was proposed as the lowest oral PDE for ptBP.

Derivation of PDE for parenteral route

The bioavailability (BA) of ptBP via oral administration was unclear. The [^{14}C]-labelled ptBP was given oral-

ly to male Wistar rats for 3 days, and the urine and feces were collected daily. The results showed 26.7% and 72.9% of the administered dose was eliminated through feces and urine, respectively (Freitag *et al*., 1982). However, in this study there was no information regarding whether the radioactivity in the feces was derived from metabolites or unabsorbed ptBP.

Efficient oral absorption of ptBP was expected because it has a low molecular weight (150.22), low Log Pow value (3.29), and high solubility in water (610 mg/L; EU Risk Assessment Report, 2008). Meanwhile, the ICH Q3D(R1) guideline recommends that, when the oral bioavailability is ≥ 50% and < 90%, the parenteral PDE can be derived by dividing the oral PDE by a modifying factor of 2 in the absence of data, and/or where data are available but are not considered sufficient for the safety assessment of the parenteral administration route.

In conclusion, the PDE for the parenteral administration route was proposed as 1 mg/day, because the BA of ptBP through oral administration was estimated to be > 50% based on the concept of the Q3D (R1) guideline.

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Conflict of interest---- The authors are employed at the company funding the research.

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