예시 1

국가·국제기구 평가보고서를 통한 시험항목의 자료제출 생략사유 및 증명자료

대상물질 : Tris(1,3-dichloro-2-p1ropyl) phosphate (CAS No.13674-87-8)
시험항목 : 저서생물 만성독성

등록제출자료 생략의 사유

(출처명) 본 생략사유 및 증명자료는 유럽연합 위해성 평가보고서(EU RAR: European Union Risk Assessment Report, 2008) 결과를 참고하였습니다.

(주요 종말점 및 결과값과 주요영향) Tris(1,3-dichloro-2-propyl) phosphate(CAS No.13674-87-8)의 저서생물 만성독성 결과, 깔따구(Chironomus riparius)의 성장 및 출현한 암수 성충의 수를 측정한 시험에서 NOEC(28일) = 3.9 mg/kg dwt(development)(GLP), EC_{50}(28일) = 16 mg/kg dwt(emergence)(GLP)로, 단각류(Hyallela azteca)의 생존 및 번식을 측정한 시험에서 EC_{50}(28일) > 71 mg/kg dwt(survival)(GLP), NOEC(28일) = 71 mg/kg dwt(survival/reproduction)(GLP)로, 실지렁이붙이(Lumbriculus variegatus)의 생존 및 번식을 측정한 시험에서 EC_{50}(28일) > 60 mg/kg dwt(survival)(GLP), NOEC(28일) = 60 mg/kg dwt(survival/reproduction)(GLP)로 기술되어 있습니다.

(생략 시험항목) 해당결과를 통해 저서생물만성독성의 유해성을 판단할 수 있으므로 화학물질의 등록 및 평가 등에 관한 법률 시행령 제13조 제6호의2에 따라 Tris(1,3-dichloro-2-propyl) phosphate(CAS No.13674-87-8)의 저서생물만성독성 자료를 생략하고자 합니다.

증명자료

생략사유의 증명자료로 아래와 같이 해당 자료의 국문요약을 참고로 제시합니다.

<표> 저서생물 만성독성 시험결과(요약)

출처: European Union Risk Assessment Report [May 2008], 83~86쪽

No.	자료개요 및 시험방법	시험결과
1	- **자료의 성격**: 주요자료, 요약서 - **신뢰도**: 신뢰도 1(valid without restriction) - **근거(인용)**: 유럽연합 위해성 평가보고서(EU RAR) 저서생물 만성독성 평가 자료로 인용 - **시험방법**: OECD Guideline 218 - **시험조건**: 28일 지수식 시험, 시험물질 첨가 퇴적물 (유기탄소 함량 5.3%)	- **종말점 및 결과값**: • NOEC(28일) = 3.9 mg/kg dwt(development) • LOEC(28일) = 8.5 mg/kg dwt(development) • EC_{50}(28일) = 16 mg/kg dwt(emergence)

No.	자료개요 및 시험방법	시험결과
	- **GLP 준수여부**: GLP 준수 - **시험물질 정보**: Tris(1,3-dichloro-2-propyl) phosphate(순도 미기재) - **시험종 정보**: 깔따구(Chironomus riparius) - **시험용량**: 기술되지 않음	
2	- **자료의 성격**: 주요자료, 요약서 - **신뢰도**: 신뢰도 1(valid without restriction) - **근거(인용)**: 유럽연합 위해성 평가보고서(EU RAR) 저서생물 만성독성 평가 자료로 인용 - **시험방법**: ASTM E 1706-00 OPPTS 850.1735 - **시험조건**: 28일 유수식 시험, 시험물질 첨가 퇴적물(유기탄소 함량 5.7%) - **GLP 준수여부**: GLP 준수 - **시험물질 정보**: Tris(1,3-dichloro-2-propyl) phosphate(순도 미기재) - **시험종 정보**: 단각류 (Hyallela azteca) - **시험용량**: 기술되지 않음	- **종말점 및 결과값**: • NOEC(28일) = 71 mg/kg dwt(survival/reproduction) • LOEC(28일) > 71 mg/kg dwt(survival/reproduction) • EC_{50}(28일) > 71 mg/kg dwt(survival)
3	- **자료의 성격**: 주요자료, 요약서 - **신뢰도**: 신뢰도 1(valid without restriction) - **근거(인용)**: 유럽연합 위해성 평가보고서(EU RAR) 저서생물 만성독성 평가 자료로 인용 - **시험방법**: ASTM E 1706-00 OPPTS 850.1735 - **시험조건**: 28일 유수식 시험, 시험물질 첨가 퇴적물(유기탄소 함량 5.7%) - **GLP 준수여부**: GLP 준수 - **시험물질 정보**: Tris(1,3-dichloro-2-propyl) phosphate(순도 미기재) - **시험종 정보**: 실지렁이붙이 (Lumbriculus variegatus) - **시험용량**: 기술되지 않음	- **종말점 및 결과값**: • NOEC(28일) = 60 mg/kg dwt(survival/reproduction) • LOEC(28일) > 60 mg/kg dwt(survival/reproduction) • EC_{50}(28일) > 60 mg/kg dwt(survival)

본 자료는 "화학물질등록평가법 시행령 제13조 및 같은법 시행규칙 제5조"에 따라 제출이 필요한 생략사유 및 증명자료의 예시로 추가검토·보완을 통해 수정·변경될 수 있으며 단순 참고자료로 활용하시기 바랍니다.

[별첨(원문 페이지 발췌)]

시험결과 표(또는 내용)

Toxicity to sediment dwelling organisms							
Chironomus riparius (midge)	28-day test based on OECD guideline 218 (February 2001)	2006	Day 0-3 NOEC development 28-day NOEC development 28-day LOEC development 28-day EC50 emergence	8.8 mg/kg dwt (3-day geometric mean measured) (13 mg/kg dwt (nominal)) 3.9 mg/kg dwt (28-day time-weighted geometric mean measured) (13 mg/kg dwt (nominal)) 8.5 mg/kg dwt (28-day time-weighted geometric mean measured) (25 mg/kg dwt (nominal)) 16 mg/kg dwt (28-day time-weighted geometric mean measured) (34 mg/kg dwt (nominal))	(1) valid without restriction	Fulfils all the reliability criteria. The study was subject to GLP. The test results are quoted in the test report relative to nominal and initial measured concentrations. For the purposes of deriving a PNEC for risk assessment the NOEC for effects on development has subsequently been recalculated on the basis of geometric mean measured concentrations for the first three days of the test. The 3-day related value is considered to cover the most susceptible first instar phase of the life-cycle of the test organisms and a period of the test when the exposure concentrations were reasonably close to the target values (see section 3.3.1.1.6 for full discussion and justification). The NOEC for effects on development and the EC50 for effects on adult emergence have also been calculated relative to 28-day time-weighted geometric mean measured concentrations. These values are included within the table along with the corresponding nominal values for comparative purposes only. The test sediment contained 5.3% total organic carbon.	Wildlife International, Ltd. (2006a).
Hyallela azteca (amphipod)	ASTM E 1706-00 OPPTS 850.1735	2006	28-day EC50 survival 28-day NOEC survival/repro 28-day LOEC	>71 mg/kg dwt (M) 71 mg/kg dwt (M)	(1) valid without restriction	Fulfils all the reliability criteria. Results are expressed relative to mean measured concentrations. The study was subject to GLP. Test sediment contained 5.7% total organic carbon.	Wildlife International, Ltd. (2006b)

Test species	Test protocol	Year test completed	Endpoint and exposure period	Result (mg/l)[1]	Reliability assessment	Comments	Study reference
			survival/repro	>71 mg/kg dwt (M)			
Lumbriculus variegatus (oligochaete)	ASTM E 1706-00 OPPTS 850.1735	2006	28-day EC50 survival 28-day NOEC survival/repro 28-day LOEC survival/repro	>60 mg/kg dwt (M) 60 mg/kg dwt (M) >60 mg/kg dwt (M)	(1) valid without restriction	Fulfils all the reliability criteria. Results are expressed relative to mean measured concentrations. The study was subject to GLP. Test sediment contained 5.7% total organic carbon.	Wildlife International, Ltd. (2006c)

Results of chronic toxicity studies carried out with three species of sediment-dwelling organisms are available:

A prolonged toxicity study with *Chironomus riparius* using spiked sediment (Wildlife International Project 583A-104), performed in April/May 2005.

A prolonged toxicity study with *Lumbriculus variegatus* using spiked sediment (Wildlife International Project 583A-106), performed in June/July 2005

A prolonged toxicity study with *Hyalella azteca* using spiked sediment (Wildlife International Project 583A-103), performed in November/December 2005

The studies were carried out using procedures that are considered to be current best practice and have been assigned reliability 1.

In all three tests, sufficient food for the duration of the test was delivered to the sediment at the start of the studies. The results of the studies are all considered to be valid without restrictions and are reported in **Table 3.34**. However there were differences in the methods and results that required consideration when deciding on the most appropriate NOEC to use as the basis for deriving a PNEC for sediment.

There were differences in the sampling and analysis methods that were used to determine exposure concentrations of TDCP in the three studies:

The analytical sampling schedule employed in the *C. riparius* study was that proposed in paragraph 38 of OECD guideline 218. Samples of overlying water, pore water and sediment were taken from the highest and lowest TDCP test concentrations at the start, on day 7 and at the end of the study. The analytical results showed an apparent dramatic decline in measured sediment concentrations from 11 and 260 mg TDCP/kg respectively at the start of the study in the lowest and highest treatments to concentrations that were below the Limit of Quantitation (LoQ, 3 mg/kg) at the end of the study. Significant concentration losses were also observed in the pore water and overlying water samples.

The sampling schedule employed in the *Lumbriculus variegatus* study was modified in the light of the *C. riparius* analytical results in order to obtain a better understanding of the stability of TDCP exposure concentrations in all treatments. All test concentrations were sampled at the start, on day 7 and at the end of the study (day 28). The resulting measured sediment concentrations showed only a limited decline over the duration of the study.

The sampling schedule was further modified for the study with *Hyalella azteca*. Samples from all test concentrations were taken at the start, on days 7, 14 and 21 and again at the end of the study (day 28). In addition, in an attempt to explain the decline in exposure concentration observed in the *C. riparius* study, a more rigorous sample extraction procedure was employed. Thus each sample was extracted three times. The first extraction was with acetone, then hexane, followed by a 50:50 mixture of acetone and hexane. The analytical results showed that, despite variability between time points and a slight decline over time, measured TDCP concentrations in sediment remained similar over a 28-day exposure period. This suggested that TDCP was not binding irreversibly to sediment, and that what was extracted on Day 0 could therefore also be extracted on Day 28. The results also showed that the additional extractions provided negligible changes in TDCP concentrations in sediment and that acetone extraction alone (as employed in the *C. riparius* and *L. variegatus* studies) would recover the TDCP in the sample.

The results of analysing exposure concentrations in the three tests suggested that there were significant declines in concentration in the *C. riparius* test that were not apparent to the same degree in the *L. variegatus* and *H. azteca* tests. The decline could be attributed in part to renewal of the overlying water, but a mass balance calculation showed this potential route of loss to be insufficient to account for all of the concentration decline. The loss could not be accounted for by the rigour of the sediment extraction procedure because the use of a more rigorous extraction regime in the *Hyalella azteca* test resulted in only a small increase in recovery. Differences in degradation or adsorption/desorption behaviour in the three test systems can also be discounted as a loss route because results of other studies have shown:

No mineralisation and no degradation of TDCP over the 122 day period of an aerobic soil degradation test in soil (see section 3.1.3.1.3).
TDCP added to anaerobic digested sludge was not biodegraded completely in sediment after 60 days (see section 3.1.3.1.2).
The adsorption/desorption behaviour of TDCP to be consistent with what would be expected on the basis of its K_{ow} value (see section 3.1.3.2.1).

The explanation for the apparent significant differences in the patterns of exposure concentration stability that were observed in the *C. riparius* test compared to the *L. variegatus* and *H. azteca* tests therefore remains unclear. In the absence of any other reasonable explanation, it is considered that the most likely cause of the apparent loss was adsorption to very fine particulates, which would have been removed during renewal of the overlying water. There was a different exposure regime used in this study compared to the *L. variegatus* and *H. azteca* tests. This is a proposal only; however, it should be noted that the study has been conducted to the best available standards and the losses from the sediment solid phase have been accounted for by the use of a geometric mean of the sediment concentrations.

Expression of no observed effect concentrations (NOECs)

In view of the apparent differences in the pattern of measured TDCP sediment concentrations in the studies it is necessary to use a different approaches to expressing the results of the *C. riparius* test compared to the *L. variegates* and *H. azteca* tests.

Chironomus riparius

The rate of *C. riparius* metamorphosis from egg, through the four larval instars and pupa stages, to adult is temperature-dependent. At 20°C, egg hatching takes approximately 5 days and the larval stages develop through their four instars over approximately 25 days. The pupa stage lasts, on average, about 10 days before emergence of the adult. A 28-day test period is sufficient for *C. riparius* to complete its development when the test is started with eggs (Taylor et al., 1991).

Watts and Pascoe (1998), McCahon and Pascoe (1991) and Ristola (2000) have reported that the first larval instar life-stage, lasting approximately three days post hatch, is the most susceptible to the effects of toxicants. The possibility of 1st instar larvae being even more susceptible to the effects of toxicants than the population as a whole has been highlighted by Ristola (2000). Increased levels of 1st instar larval mortality arising from the effects of toxic substances may be compensated for by lower levels of mortality in subsequent life-stage because of density-dependent factors such as food availability.

In view of the above and the fact that the early days of the test also corresponded with the period when there was likely to have been closest agreement between target and actual exposure concentrations, the NOEC for the test has been expressed relative to estimated geometric mean concentrations over the first 3 days. This provided a NOEC that, because of the known susceptibility of the 1st instar larvae, was likely to be close to and probably no higher than the NOEC for the whole life-cycle. This practice represented a divergence from the OECD guideline but was considered to provide a NOEC that, given the uncertainties in the exposure concentrations during the test, was based on the most reasonable interpretation of the data relative to the properties of the test substance.

The estimation of geometric mean concentrations over the first 3 days required extrapolating from the patterns of loss apparent in measured concentrations in the lowest (13 mg/kg nominal) and highest (200 mg/kg nominal) treatments between day 0 and day 7 of the test assuming a logarithmic decline in concentration over this period. For the 10.6 and 268 mg/kg day 0 measured concentrations the corresponding estimated day 3 concentrations are 7.3 and 190 mg/kg. From these were calculated geometric mean concentrations for the day 0 to day 3 period of the test of 8.8 and 226 mg/kg. If necessary, for example to calculate an EC_{50}, the concentrations corresponding to the intermediate treatments could be determined by interpolation but in this test it was not necessary to do this because the NOEC corresponded to the lowest treatment.

For comparative purposes only the NOEC was also determined relative to time-weighted geometric mean measured concentrations over the 28-day test period. These were calculated from measured concentrations on days 0, 7 and 28 of the test using methods described in OECD guidance (OECD, 2000).

The resulting NOEC determined from the geometric mean concentrations over the first 3 days is 8.8 mg/kg dw (equivalent to 8.3 mg/kg dw and 1.8 mg/kg wwt in a standard sediment containing 5% organic matter) based on the 3-day geometric mean concentration. For comparative purposes, the value based on the geometric mean measured concentrations over the 28 day test period is 3.9 mg/kg dw (equivalent to 3.68 mg/kg dw and 0.8 mg/kg wwt in a standard sediment containing 5% organic matter).

Lumbriculus variegatus and Hyalella azteca

The results of the L. variegatus and H. azteca studies have been expressed relative to time-weighted geometric mean measured concentrations over the duration of the test. This approach is recommended by the OECD (OECD, 2000). The measured concentrations determined in these two studies were similar in terms of decline over time and variability and were therefore considered appropriate and reliable for such a method of expression.

NOECs for survival and reproduction of 60 and 71 mg/kg dw (equivalent to 53 and 62 mg/kg dw and 11 and 13 mg/kg wwt in a standard sediment containing 5% organic matter) have been determined for L. variegatus and H. azteca respectively.