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대체시험자료 활용 안내서

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▶ 본 안내서는 화학물질의 등록 및 평가 등에 관한 법률(이하 “화평법”)에서 규정하고 있는 등록을 위한 제출자료 준비 시 다양한 대체시험자료를 활용하기 위한 절차, 방법 등에 대해 설명한다.

▶ 본 안내서는 화학물질 등록서류 준비를 원활하게 이행할 수 있게 하기 위한 목적으로 작성하였으며, 다양한 활용 예시를 제공하여 화학물질의 등록, 유해성 심사 관련 절차를 이해하기 위한 목적으로 활용할 수 있다.

- 목 차 -

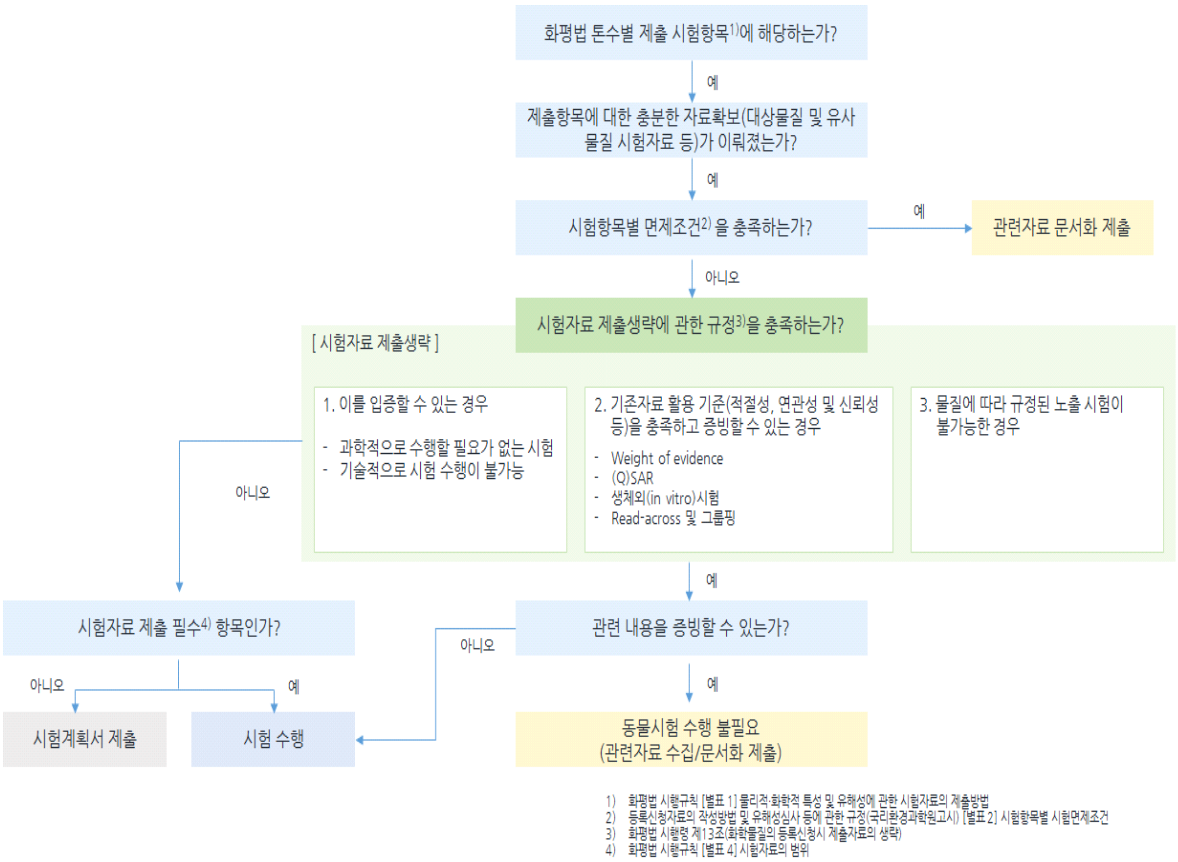
1. 목적 및 개요

1.1. 목적

본 안내서의 목적은 “화학물질의 등록 및 평가 등에 관한 법률 (이하, 화평법)” 에 따라 등록하고자 하는 경우 시험자료 준비에 있어 동물실험자료를 대신하여 화학물질에 대한 유해성 등을 확인할 수 있는 대체시험자료로 활용하는 방법 및 절차 등을 제공하는 것이다.

또한 시험자료 대체방법의 활용 예시를 제공함으로써 산업계가 환경부에 제출하기 위한 자료준비에 적절한 대응이 가능하도록 참고자료로 이용될 수 있다.

1.2 개요



[그림 1] 자료제출 적용에 대한 의사결정 절차

화학물질 등록신청인은 화평법 시행규칙 [별표 1] 제1호부터 제7호까지의 연간 제조·수입량 범위에 따라 요구되는 시험자료를 제출해야 한다. 다만 같은 법 시행규칙 [별표 3의 3] 제1호·제2호에 따라 등록신청 시 제출자료가 생략되는 기존화학물질은 시험자료 간소화가 적용되며, 고분자화합물이나 현장분리중간체 또는 수송분리중간체에 대해서도 특례가 적용되므로 제조·수입량, 분류·표시 결과, 고분자화합물, 노출이 통제된 사용 등 여부 등에 따라 제출하는 시험자료는 달라질 수 있다.

특히 화학물질의 특성, 유해성, 취급조건, 노출형태에 따라서 시험자료가 생략되는 경우가 있으므로 화평법 시행규칙 [별표 1]에 따라 제출해야 하는 시험자료는 등록·평가와 관련하여 화평법에서 규정하고 있는 모든 요구사항을 전반적으로 고려해야 한다.

등록신청인은 제조·수입량에 따라 화평법에서 요구하고 있는 시험자료 범위와 관계없이 가용한 모든 정보를 수입하는 것이 필요하다. 이를 통해 화학물질의 유해성, 분류·표시를 확인할 수 있으며 시험자료 간소화 적용대상 유무도 확인할 수 있다. 특히 문헌자료 등 공개 자료를 통해 등록에 필요한 새로운 동물실험을 생략할 수 있는데, 이러한 자료가 대체시험자료(QSAR, read-across, WoE, *in vitro* 등)의 중요한 정보원으로 활용될 수가 있기 때문이다.

[1단계: 기존 정보 수집 및 자료공유]

등록신청인은 등록대상 화학물질에 대해 공개된 문헌자료를 포함하여 가용한 모든 시험자료를 수집해야 한다. 추가적으로 화평법 시행규칙 [별표 2]와 [별표 8]의 화학물질의 용도에 따른 노출, 위해성 관리대책에 대한 정보를 수집해야 한다. 기존화학물질에 대한 시험자료의 경우 화평법 제15조에 따라 대표자를 정하여 공동으로 제출하도록 하고 있으므로 기존화학물질은 협의체 구성원의 물질에 대한 용도와 노출 정보를 함께 고려해야 한다.

등록신청인은 이러한 모든정보를 함께 고려해야 등록에 필요한 추가 자료를 생산·확보 여부를 결정할 수 있다.

GLP(Good Laboratory Practice)에서 발행한 시험보고서(Test report) 또는 이를 바탕으로 작성한 요약보고서(RSS, Robust Study Summaries)가 아닌 자료를[1] 제출하고자 할 경우에는 다음의 원칙을 참고하여 자료를 제출해야 한다.

1) 1. 저널(학술논문), 과학 핸드북, 독성 DB와 같은 문헌자료, 2. 국제기구나 선진국의 화학물질 평가 보고서로 구분 가능

[표 3] 문헌자료 및 국외 평가보고서

구분	예시
문헌자료	- 특정한 화학물질(군)에 대한 시험결과를 발표한 학술 논문 - 전문가 그룹에서 통용되며, 학술적으로 널리 인정되는 시험결과를 수록한 과학 핸드북(Merk index 등) - 학술 논문, 과학 핸드북, 특정 기업이나 국가 보유의 시험결과를 전문가가 검증·통합 제공하는 독성 데이터베이스(DB)
평가보고서	- OECD, WHO 등 국제기구에서 평가한 보고서 (예, OECD SIDS, IARC monograph 등) - EU, 미국 등 선진국의 화학물질 관리를 위한 국가보고서 (예, EU RAR(Risk Assessment Report, 위해성평가보고서), 미국 RED(Reregistration Eligibility Decision, 농약재등록평가보고서) 등)

수집한 문헌자료가 아래의 세 가지 경우에 해당한다면 등록 자료로 제출할 수 있다.

1) 시험항목에 대한 유해성을 확인할 수 있는 시험결과(시험방법, 결과 값 및 결론)가 상세히 기술되어 있고, 국제적으로 인정되는 시험방법에 따라 수행된 신뢰성이 있는 시험결과인 것으로 판단되는 경우

2) 시험항목에 대한 시험결과를 유해성 또는 위해성 평가에 핵심자료로 활용하였거나 GHS 등에 따른 유해성 분류에 활용한 경우
   - 화평법 시행령 제13조 제6호에 따른 시험자료 생략 규정을 적용하기에 적합한 것으로 판단되는 자료(일반적으로 국가 평가보고서의 관련 항목을 조합하여 근거자료로 제출하는 경우)

3) 물리·화학적 특성에 대한 시험결과를 모아 수록한 과학 핸드북으로, 해당 과학 분야에서 널리 통용되어 받아들여지는 시험결과(Merk index, pesticide manual, HSDB 등)

단순히 시험결과 값만 제시되어 있는 문헌자료는 유해성 분류, 용량-반응 결정 등 유해성평가가 어려우므로 일반적으로 인정되지 않는 자료라 할 수 있다.

[2단계: 제출 시험자료 확인]

등록신청인은 등록을 위해 어떠한 시험자료가 필요한지 확인해야 한다. 제출하는 시험자료는 시행규칙 [별표 1](물리적·화학적 특성 및 유해성에 관한 시험자료의 제출방법)의 제조·수입량 톤수 구분에 따라 달리 요구되고 있으나, 아래의 1) 또는 2)에 해당하고 그 정당성을 충분히 입증하여 관련 자료를 함께 제출하는 경우에는 해당 항목의 시험자료 제출이 생략 가능하다.

1) 기술적으로 시험이 불가능한 물질 또는 과학적으로 시험이 불필요한 물질
   - 화평법 시행령 제13조제7호 및 국립환경과학원고시(등록신청자료의 작성방법 및 유해성심사 방법 등에 관한 규정 [별표 2])에 해당
   - [표 2]와 같은 시험면제조건에 해당하는 화학물질의 경우에 해당 시험항목에 대한 새로운 시험은 불필요

[표 4] 유해성 시험항목별 시험항목 생략조건(물질의 특성 및 유해성에 근거)

【인체 유해성 시험항목】		
시험항목		**시험면제조건**
급성독성	급성경구독성	- 피부 부식성으로 분류되는 물질
	급성경피독성 또는 급성흡입독성	- 피부 부식성으로 분류되는 물질 - 입자의 크기가 100㎛를 초과하는 물질. 단, 급성흡입독성 시험에 한함
자극성	피부 자극성/부식성	- 기존의 가용한 정보에 근거할 때 피부 부식성으로 분류되는 물질 - 강산(pH ≤ 2.0) 또는 강염기(pH ≥ 11.5) 물질로 피부 부식성으로 분류되는 물질 - 상온에서 인화성 물질(공기 중에서 자연 발화하는 경우) - 급성경피독성 시험에서 고독성인 물질(구분 1 또는 구분 2) - 용량 2,000mg/kg 이상에서 수행된 급성경피독성 시험에서 피부 자극성이 나타나지 않는 물질
	눈 자극성/부식성	- 기존의 가용한 정보에 근거할 때 피부 부식성이나 심한 눈 손상 물질로 분류되는 물질 - 강산(pH ≤ 2.0) 또는 강염기(pH ≥ 11.5) 물질로 피부 부식성으로 분류되는 물질 - 상온에서 인화성 물질(공기 중에서 자연 발화하는 경우)
과민성	피부 과민성	- 피부 부식성이나 피부 과민성으로 분류되는 물질 - 상온에서 인화성 물질(공기 중에서 자연 발화하는 경우)
유전독성	포유류 배양세포를	- *In vivo* 체세포 염색체이상 시험자료가 있는 물질 - 발암성(구분 1) 또는 생식세포 변이원성(구분 1, 2)으로 알려진 물질

	이용한 염색체이상	
	시험동물을 이용한 유전독성	- 제조·수입량이 100톤 미만인 경우로, 복귀돌연변이시험 및 *in vitro* 염색체이상시험이 모두 음성인 물질 - 포유류 배양세포를 이용한 유전자변이 시험자료가 있는 물질. 단, 복귀돌연변이시험 및 *in vitro* 염색체이상시험이 모두 음성인 경우에 한함 - 발암성(구분 1) 또는 생식세포 변이원성(구분 1, 2)으로 알려진 물질
	추가 유전독성(생식세포 유전독성 등)	- 복귀돌연변이시험, *in vitro* 염색체이상시험 및 *in vivo* 유전독성시험 결과 유전독성물질 분류(생식세포 변이원성 구분 1 및 2)를 위한 추가적인 평가가 필요 없다고 판단되는 물질
반복투여(노출) 독성	반복투여독성(28일)	- 반복투여독성(90일) 또는 만성독성 시험자료가 있는 물질 - 즉시 분해되고 분해산물에 대한 충분한 자료가 있는 물질
	반복투여독성(90일)	- 반복투여독성(28일)에서 중대한 독성이 관찰된 28일 기준값을 적절한 불확실성 계수를 적용하여 90일 값으로 외삽 적용한 경우 특정 표적장기독성(반복 노출)으로 분류되는 물질 - 즉시 분해되고 분해산물에 대한 충분한 자료가 있는 물질 - 반응성이 없고 불용성이며, 흡입할 수 없고 흡수되지 않으며, 28일 한계시험에서 독성 증거가 없는 물질
생식·발달 독성	생식 및 발달(발생)독성 스크리닝	- 최기형성 또는 2세대 생식독성 시험자료가 있는 물질 - 확장 1세대 생식독성 시험자료가 있는 물질
	최기형성	- 태아 발달 독성을 일으키는 것으로 알려진 생식독성물질(구분 1)
	2세대 생식독성	- 생식능력에 손상을 일으키는 것으로 알려진 생식독성물질(구분 1) - 확장 1세대 생식독성 시험자료가 있는 물질
발암성	발암성	- 생식세포 변이원성(구분 1)으로 분류되는 물질(발암성이 유전독성 메카니즘으로 일어난다고 간주되는 경우) - 국제암연구소 등 국제적전문기관의 발암 평가보고서에 근거할 때 발암성으로 분류되는 물질 - 발암성(구분 1)으로 분류되는 물질 - 생식세포 변이원성(구분 2)으로 분류되지 않고, 반복투여(노출) 시험결과 증식 또는 선종양을 일으킨다는 증거가 없는 물질
【환경 유해성 시험항목】		
시험항목		**시험면제 조건**
수생생물 독성	어류급성독성	- 생체막을 통과할 수 없는 등 수생생태독성이 없다는 증거가 있는 물질 - 어류에 대한 만성독성 시험자료가 있는 물질
	물벼룩급성독성	- 생체막을 통과할 수 없는 등 수생생태독성이 없다는 증거가 있는 물질 - 물벼룩에 대한 만성 수생독성 시험자료가 있는 물질

	담수조류 성장저해	- 생체막을 통과할 수 없는 등 수생생태독성이 없다는 증거가 있는 물질
침전물 독성	활성슬러지 호흡저해	- 물에 극히 불용성 등 미생물 독성이 발생하지 않을 것 같은 완화 요건이 있는 물질
분해성	이분해성	- 무기물
	본질적 분해성	- 무기물 - 이분해성 물질 - 모의 생분해성 시험자료(지표수, 토양, 수중 퇴적물)가 있는 물질
	pH에 따른 가수분해	- 이분해성 또는 본질적분해성 물질 - 물에 극히 불용성인 물질
	분해산물의 확인	- 이분해성 또는 본질적분해성 물질
	환경 거동 및 동태에 대한 추가정보	- 무기물 - 이분해성 또는 본질적분해성 물질
생물 농축성	생물농축성	- 생물농축 가능성이 낮거나(예, logKow < 3), 생체막을 통과할 수 없다고 예상되는 물질 - 이분해성 또는 본질적분해성 물질 - 무기물
흡착/ 탈착	흡착 및 탈착	- 물리화학적 성질에 근거하여, 흡착 가능성이 낮은 물질 (예, 비이온성 물질로 logKow < 3) - 물질 및 그 분해산물이 빠르게 분해되는 물질 - 신뢰성 있는 스크리닝 방법 등을 통해 흡착 및 탈착을 추정할 수 있는 물질
	흡착 및 탈착에 대한 추가 정보	- 물리화학적 성질에 근거하여, 흡착 가능성이 낮은 물질 (예, 비이온성 물질로 logKow < 3) - 물질 및 그 분해산물이 빠르게 분해되는 물질 - 흡착 및 탈착에 관한 측정 시험자료가 있는 물질

2) 화학물질의 노출시나리오 등 용도와 관련한 노출 정보에 근거하여 시험이 생략되는 물질

- 화평법 시행령 제13조제8호 및 국립환경과학원고시(등록신청자료의 작성방법 및 유해성심사 방법 등에 관한 규정 [별표 2])에 해당
- [표 3]과 같은 시험면제조건에 해당하는 화학물질의 경우에 해당 시험항목에 대한 새로운 시험은 불필요

[표 5] 유해성 시험항목별 시험항목 생략조건(노출시나리오에 근거)

【인체 유해성 시험항목】		
시험항목		**시험면제조건**
급성독성	급성경구독성	- 물리적·화학적 특성이나 용도상으로 주된 노출경로가 흡입으로 판단되어 급성흡입독성 시험자료를 제출하는 물질
반복투여(노출) 독성	반복투여독성(28일)	- 노출시나리오에 따라 인체 노출이 배제될 수 있는 물질
생식·발달독성	생식 및 발달(발생)독성 스크리닝	- 유전독성 발암물질(생식세포 변이원성 구분 2이며, 발암성 구분 1)로 알려져 있고 적절한 위해성관리대책이 이행되는 물질 - 생식세포 변이원성물질(구분 1)로 알려져 있고 적절한 위해성관리대책이 이행되는 물질 - 노출시나리오에 따라 인체 노출이 배제될 수 있는 물질
	최기형성	- 유전독성 발암물질(생식세포 변이원성 구분 2이며, 발암성 구분 1)로 알려져 있고 적절한 위해성관리대책이 이행되는 물질 - 생식세포 변이원성물질(구분 1)로 알려져 있고 적절한 위해성관리대책이 이행되는 물질 - 독성학적 활성도가 낮고 인체에 대한 노출을 무시할 수 있는 물질
	2세대 생식독성	- 유전독성 발암물질(생식세포 변이원성 구분 2이며, 발암성 구분 1)로 알려져 있고 적절한 위해성관리대책이 이행되는 물질 - 생식세포 변이원성물질(구분 1)로 알려져 있고 적절한 위해성관리대책이 이행되는 물질 - 독성학적 활성도가 낮고 인체에 대한 노출을 무시할 수 있는 물질
발암성	발암성	- 광범위하게 분산되는 용도로 사용되지 않고 인체 노출 빈도가 낮으며, 인체에 장기간 노출되지 않는 물질
【환경 유해성 시험항목】		
시험항목		**시험면제조건**
수생생물 독성	어류만성독성	- 위해성에 관한 자료 작성 결과, 수생 생물에 대한 화학물질의 영향을 추가적으로 조사할 필요가 없다는 증거가 있는 물질
	물벼룩만성독성	- 위해성에 관한 자료 작성 결과, 수생 생물에 대한 화학물질의 영향을 추가적으로 조사할 필요가 없다는 증거가 있는 물질
육상생물 독성	육생식물 급성독성	- 토양 노출이 무시할 만한 수준인 물질 - 평형분배방법에 의한 토양생물 유해성평가가 가능한 물질
	육생 무척추동물 급성독성	- 토양 노출이 무시할 만한 수준인 물질 - 평형분배방법에 의한 토양생물 유해성평가가 가능한 물질
	육생식물 만성독성	- 토양 노출이 무시할 만한 수준인 물질 - 위해성에 관한 자료 작성 결과, 육상 생물에 대한 화학물질의 영향을 추가적으로 조사할 필요가 없다는 증거가 있는 물질

	육생 무척추동물 만성독성	- 토양 노출이 무시할 만한 수준인 물질 - 위해성에 관한 자료 작성 결과, 육상 생물에 대한 화학물질의 영향을 추가적으로 조사할 필요가 없다는 증거가 있는 물질
침전물 독성	활성슬러지 호흡저해	- 하수처리시설로 배출되지 않는 물질 - 이분해성물질로 시험에 사용된 농도가 하수처리 시설로의 예상 유입 농도의 범위 내인 물질
	저서생물 만성독성	- 위해성에 관한 자료 작성 결과, 저서생물에 대한 화학물질의 영향을 조사할 필요가 없다는 증거가 있는 물질
분해성	본질적 분해성	- 위해성에 관한 자료 작성 결과, 화학물질의 분해성을 추가적으로 조사할 필요가 없다는 증거가 있는 물질
	pH에 따른 가수분해	- 위해성에 관한 자료 작성 결과, 화학물질의 분해성을 추가적으로 조사할 필요가 없다는 증거가 있는 물질
	분해산물의 확인	- 위해성에 관한 자료 작성 결과, 화학물질의 분해산물을 확인할 필요가 없다는 증거가 있는 물질
	환경 거동 및 동태에 대한 추가정보	- 위해성에 관한 자료 작성 결과, 화학물질의 환경거동 및 동태를 추가적으로 조사할 필요가 없다는 증거가 있는 물질

3) 비소비자용이면서 유해성이 없거나 낮은 유해성으로 분류되는 물질

- 아래의 유해성분류에만 해당되는 물질: 톤수에 무관하게 1~10톤 자료만 제출

유해성 항목		분류
1	급성독성	분류되지 않거나 근거자료 없음 (제출자료 범위 밖)
2	피부 부식성 또는 자극성	
3	심한 눈 손상 또는 눈 자극성	
4	호흡기 또는 피부 과민성	
5	생식세포 변이원성	
6	발암성	
7	생식독성	
8	특정 표적장기 독성 (1회)	
9	특정 표적장기 독성 (반복)	
10	흡인 유해성	
11	수생환경 급성 유해성	
12	수생환경 만성 유해성	구분 3, 4
13	오존층 유해성	분류되지 않거나 근거자료 없음 (제출자료 범위 밖)
	위해성에 관한 자료	생략

- 아래의 유해성으로만 분류되고 그 밖의 인체나 환경유해성(구분 3, 4 제외)으로 분류되지 않는 물질: 100톤 이상이 되어도 환경유해성 자료는 100톤 미만 5개 자료만 제출

유해성 항목		분류
1	급성독성	구분 4
2	피부 부식성 또는 자극성	구분 2
3	심한 눈 손상 또는 눈 자극성	구분 2
4	호흡기 또는 피부 과민성	피부 구분 1
5	생식세포 변이원성	-
6	발암성	-
7	생식독성	추가 구분
8	특정 표적장기 독성 (1회)	구분 3
9	특정 표적장기 독성 (반복)	-
10	흡인 유해성	-
11	수생환경 급성 유해성	-
12	수생환경 만성 유해성	-
13	오존층 유해성	구분 1
	위해성에 관한 자료	제출

[3단계: 대체자료 활용]

화평법 시행령 제13조에 의하면 동물시험을 대신하여 시험관내 시험법(제4호)의 시험 값이나 QSAR(제3호) 또는 read-across 접근법(제5호)으로 유사한 다른 물질의 정보로부터 얻은 독성 예측값을 제출할 수 있다. 또한 기술적으로 시험이 불가능하거나(제7호), 외국정부 또는 국제기구에서 공개한 유해성 평가결과(제6조의2), 기타 다른 신뢰성 있는 결과(제6호)를 통해 시험자료 제출을 면제받거나 다른 독성/면제신청에 대한 증거로 제출할 수도 있다.

- 활용 가능한 기존 대체 자료의 확인(예. WoE, QSAR, 생체외 시험(*in vitro*), read-across 시험 등)
- 시험자료의 적절성과 연관성 및 신뢰성을 검토한 후 기존 자료로 대체 가능

[4단계: 시험자료 생산 또는 시험계획서]

면제 또는 생략조건에 해당하지 않고, 대체자료 활용이 불가능한 경우 새로운 시험자료를 생산해야 하나, 일부 시험항목의 경우에는 시험계획서로 제출할 수 있다. 시험자료 제출 및 시험계획서 작성 항목은 구분되어 있으므로 항목별 확인이 필요하다. 시험계획서로 대체하여 제출할 수 있는 시험자료는 아래 [표 4]와 같으며, 화평법 시행규칙 [별표 4](시험자료의 범위)를 참고하여 확인할 수 있다.

[표 6] 시험계획서 제출 가능 항목

물리적 · 화학적 항목	인체 유해성 항목	환경 유해성 항목
가) 점도 나) 해리상수	가) 추가 유전독성 나) 반복투여독성(90일) 다) 생식 및 발달독성 스크리닝 라) 최기형성 마) 2세대 생식독성 바) 발암성 사) 급성흡입독성	가) 어류만성독성 나) 물벼룩만성독성 다) 육생식물 급성독성 라) 육생 무척추동물 급성독성 마) 육생식물 만성독성 바) 육생 무척추동물 만성독성 사) 활성슬러지 호흡저해 아) 저서생물 만성독성 자) 본질적 분해성 차) 분해산물의 확인 카) 환경 거동 및 동태에 대한 추가 정보 타) 생물농축성 파) 흡착 및 탈착 하) 흡착 및 탈착에 대한 추가 정보

2. 대체시험자료 활용 절차 및 원칙

2.1. 대체시험자료 활용 절차

가. 시험면제

1) 시험면제조건

기술적으로 시험을 수행하는 것이 불가능하거나 과학적으로 시험을 수행할 필요가 없는 경우, 또는 시험항목별 면제조건을 충족하는 경우 등 시험수행의 여부를 사전에 판단하여 생략하는 방법을 시험면제 또는 생략(Data waiving)이라고 한다.

등록하고자 하는 화학물질이 물질 고유의 특성이나 유해성으로 인해 시험수행이 불가능하거나 불필요한 경우(피부 부식성 물질, 인화성 물질, 불용성 물질 등 다양하며 유해성시험 항목별로 다름)가 있다. 이러한 면제조건은 일반적으로 [표 2]와 같이 국립과학원고시(등록신청자료의 작성방법 및 유해성심사 방법 등에 관한 규정)의 [별표 2]로 규정하고 있으나, 개별 화학물질에만 특정되는 다양한 면제사례도 발생할 수 있다.

등록 화학물질의 특정 시험항목에 대해 시험을 생략하기 위해서는 해당 면제에 대해 과학적으로 유효한 정당성을 입증해야 한다. 특히 [표 3]과 같이 사용 용도에 따른 노출 또는 위해성관리대책에 근거하는 경우에는 노출시나리오를 포함한 위해성에 관한 자료 등과 같은 기술 문서를 작성하여 제출해야 한다.

시험 면제를 적용할 때에는 기술 문서와 더불어 시험 생략에 대한 정당성을 입증해야 하며, 참고할 수 있는 증거자료 또한 제출하여 해당 자료에 대한 유효성을 독립적으로 평가할 수 있도록 조치해야 한다. 향후 이러한 제출자료의 기술 내용에 따라 후속 관리조치가 뒤따르게 될 것이며, 이를 통해 보다 안전한 화학물질의 사용이 권고될 수 있을 것이다.

화평법 시행령 제13조(화학물질의 등록신청시 제출자료의 생략)에는 다음에 해당하는 물질은 등록시 시험자료를 제출하지 않아도 된다고 규정되어 있다.

[화평법 시험자료 제출 면제 조건]

구조 활성관계 예측 프로그램(QSAR)

- 제3호. 제조·수입하려는 화학물질의 양이 연간 10톤 미만으로서 국제적으로 인정된 구조 활성관계 예측 프로그램(QSAR: qualitative or quantitative structure activity relationship models)로부터 얻어진 결과를 통하여 사람의 건강이나 환경에 대한 유해성을 판단할 수 있는 화학물질

생체외(*In vitro*) 시험

- 제4호. 국제적으로 인정된 시험관내 시험방법으로 얻은 결과를 통하여 사람의 건강이나 환경에 대한 유해성을 판단할 수 있는 화학물질

상관성 방식(Read-across), 증거력 방식(WoE, Weight of Evidence)

- 제5호. 구조가 유사한 화학물질로부터 얻어진 결과를 통하여 사람의 건강이나 환경에 대한 유해성을 판단할 수 있는 화학물질
- 제6호. 국제적으로 인정된 시험방법과 동등한 수준의 신뢰성이 있는 결과를 통하여 사람의 건강이나 환경에 대한 유해성을 판단할 수 있는 화학물질

물질 특성·유해성, 노출에 근거한 면제

- 제7호. 기술적으로 시험이 불가능한 화학물질
- 제8호. 화평법 제14조제1항제7호 또는 제9호에 따른 등록신청자료를 통하여 사람이나 환경에 노출되지 아니할 것으로 판단할 수 있는 화학물질

2) 톤수별 제출 시험항목 면제

인체 유해성 항목의 예를 보면 급성독성(경구 및 경피), 피부/눈 자극성 및 부식성, 과민성, 유전독성(복귀돌연변이, 염색체이상, 시험동물을 이용한 유전독성), 반복투여독성(28일) 등은 등록 시 필수 제출항목이며, 그 밖에 유해성 항목은 시험계획서로 대체하여 제출이 가능하다. 톤수별로 제출해야 하는 시험항목 중 인체 유해성 항목은

[표 5]와 같으며, 시험항목에 대한 세부 설명은 ‘화학물질의 위해성에 관한 자료 작성 지침(2017, 2021)’에 기술되어 있다.

「등록신청자료의 작성방법 및 유해성심사 방법 등에 관한 규정(국립환경과학원고시)」 [별표 2]의 시험면제 조건에 해당하는 경우, 시험자료 생략이 가능하다. 다만, 시험자료를 필수로 제출해야 하는 경우라도 새로운 시험을 수행하기 전에 대체시험자료 활용이 가능한지를 먼저 검토하는 것이 필요하다. 대체시험자료는 QSAR와 상관성 방식(Read-across) 및 생체외 시험(*in vitro*) 등을 통해 생산되며, 이때 해당 자료의 신뢰도에 따라 가중치(weight)를 부여하고 증거력 방식(WoE, Weight of Evidence)를 적용함으로써 주요자료를 선정할 수 있게 된다.

[표 7] 톤수별 인체 유해성 시험항목(예시)

대그룹	중그룹*	0.1~1톤	1~10톤	10~100톤	100~1,000톤	1,000톤 이상
급성 독성	급성 경구독성 (OECD TG 420/423/425)	○	○	○	○	○
	급성 경피독성 (OECD TG 402)			○	○	○
	급성 흡입독성 (OECD TG 403)			●	●	●
자극성 및 부식성	피부 자극성/부식성 (OECD TG 404/430/431/435/439)		○	○	○	○
	눈 자극성/부식성 (OECD TG 405/437/438)			○	○	○
과민성	피부 과민성 (OECD TG 406/429/442)		○	○	○	○
변이원성	복귀돌연변이 (OECD TG 471)	○	○	○	○	○
	포유류 배양세포를 이용한 염색체이상 (OECD TG 473/487)			○	○	○
	시험동물을 이용한 유전독성 (염색체이상 OECD TG 474/475, 유전자 변이 OECD TG 488, DNA 손상/복구 OECD TG 486/489)			○	○	○
	추가 유전독성 (생식세포 OECD TG 478/483/488)				●	●
반복 투여 독성	반복투여독성(28일) (OECD TG 407, 410, 412)			○	○	○
	반복투여독성(90일) (OECD TG 408/409, 411, 413)					●
발암성	발암성 (OECD TG 451/453)					●
생식/ 발달 독성	생식 및 발달독성 스크리닝 (OECD TG 421/422)			●	●	●
	최기형성 (OECD TG 414)					●
	2세대 생식독성 (OECD TG 416/443)					●

○: 필수 시험항목, ●: 시험계획서 제출항목 (향후 시험자료 제출 필요)
*시험항목별로 가능한 OECD 시험가이드라인(TG)을 기재(*in vitro* 등 동물대체시험방법 포함)

톤수별 제출 시험항목에서 제시하고 있는 특정 시험면제 조건에 해당되는 경우, 대부분 각 항목별 시험은 다음과 같은 면제 절차를 거치게 된다.

(i) 시험 생략
(ii) 다른 정보(기존 정보 또는 생성 예정)로 대체
(예, 단기 28일 반복독성 시험은 좀 더 신뢰성 있는 아만성 90일 반복독성 시험으로 대체될 수 있음)
(iii) 후속 단계에서 제공
(iv) 기타 다른 방법에서 채택
(예, 연간 10톤 이상 100톤 미만 제조·수입되는 물질의 급성독성 세부규칙에 의하면, 흡입 및 피부 경로를 통한 급성독성의 경우 한 가지 이상의 노출 경로 정보가 제시되어야 하며, 두 번째 노출 경로의 선택은 물질의 성질 또는 인간에게 노출 가능성이 있는 경로에 의해 결정됨)

톤수별 요구되는 정보에 따라 다양한 시험면제 사례가 있으며, 그 예시는 다음과 같다.

[예시]

1. 화학물질이 상온의 공기 중에서 자연발화성을 가지고 있을 때, 피부 자극성/부식성과 피부 과민성 시험(1톤 이상) 및 심한 눈 손상/눈 자극성(10톤 이상) 시험은 수행할 필요가 없다.

2. 화학물질이 피부 부식성(구분 1)으로 분류될 경우, 급성독성시험을 생략할 수 있다. 개정된 급성 독성 시험 조건에 따르면, 화학물질이 급성독성 또는 피부 경로를 통한 독성 시험은 수행할 필요가 없으며, 이때 추가적인 참고자료를 제출해야 한다.

3. 신뢰성 있는 단기 독성시험(28일) 자료를 가용할 수 있고, 특정표적장기독성(반복노출)물질로 분류될 정도로 심각한 건강 영향을 초래하는 화학물질인 경우, 아만성 독성시험(90일)을 수행할 필요가 없다. 이는 동일 경로에 대해 외삽법을 통해 불확실성 계수를 적용한 28일 NOAEL을 사용하여 90일 NOAEL을 추정할 수 있기 때문이다.

*국립환경과학원고시(등록신청자료의 작성방법 및 유해성심사 방법 등에 관한 규정)의 [별표 2] 시험항목별 시험면제조건 참고

나. 의사결정 절차

톤수별 제출 시험항목에 대해 시험면제 조건을 적용하기 위한 의사결정 절차는 다음과 같다.

1) 톤수별 제출 시험항목 해당 여부 확인

톤수별 제출 유해성 시험자료의 범위는 화평법 시행규칙의 [별표 1](물리적·화학적 특성 및 유해성에 관한 시험자료의 제출방법)을 참고하여 확인할 수 있다.

2) 시험항목별 면제조건 확인

시험항목별 면제조건에 대한 정보는 「등록신청자료의 작성방법 및 유해성심사 방법 등에 관한 규정」의 [별표 2] 시험항목별 시험면제조건(국립환경과학원고시)을 참고하여 확보할 수 있다.

3) 일반 규칙 준수여부 확인

시험항목별 면제조건을 충족하지 않는 경우, 시험면제에 대한 일반 규칙 준수 여부를 확인한다. 화평법 시행령 제13조(화학물질의 등록신청시 제출자료의 생략)에서는 다음에 해당하는 물질은 등록시 시험자료를 제출하지 않아도 된다고 규정하고 있다.

(i) 과학적으로 수행할 필요가 없는 시험의 경우, 시험자료의 적절성과 연관성 및 신뢰성을 검토한 후 기존 자료로 대체 가능
(ii) 동물대체시험(예, WoE, QSAR, 생체외 시험, read-across 시험 등)으로 대체가 가능한 시험
(iii) 기술적으로 시험 수행이 불가능하거나 물질에 따라 시험수행이 불필요한 경우

4) 시험자료 제출 필수항목 여부 확인

시험계획서로 대체하여 제출할 수 있는 시험자료는 화평법 시행규칙 [별표 4](시험자료의 범위)를 참고하여 확인할 수 있다.

[예시]

화평법 시행령 제13조에 의하면 동물시험을 대신하여 시험관내 시험법(제4호)으로부터 얻은 시험결과나, QSAR(제3호) 또는 read-across 접근법(제5호)으로 유사한 다른 물질의 정보로부터 얻은 독성 예측결과를 대신 제출할 수 있다. 또한 기술적으로 시험이 불가능하거나(제7호), 다른 신뢰성 있는 결과(제6호)를 통해 시험자료 제출을 면제받거나 다른 독성/면제신청에 대한 증거로 제출할 수도 있다.

같은 법 시행령 제13조 제3호에 따르면 QSAR 결과를 제출할 수 있는 경우는 제조·수입량이 연간 10톤 미만인 화학물질로 제한하고 있다. 이는 제조·수입량이 10톤 미만인 등록물질에 대해 요구되는 자료를 의미하는 것으로, 즉 10톤 이상을 초과하는 제조·수입량에 대해서는 급성 경구독성 또는 어류 급성독성 등과 같은 시험자료를 대신하여 QSAR 자료를 단독으로는 제출할 수 없다는 것을 의미한다. 기존 시험자료의 신뢰도가 충분히 높지 않거나 구조 유사물질의 시험자료를 활용할 때, 증거력 방식의 보충자료로 QSAR 자료를 활용할 때는 등록 톤수와 관계없이 제출할 수 있다.

다음 표에 제시된 항목은 QSAR 프로그램에 적용되는 항목으로 물리적·화학적 성질, 인체, 생태독성 및 환경거동의 특성을 예측하는 목적으로 사용되고 있다. 화평법 제14조 및 시행령 13조 제3호에 따라 다음에 해당되는 시험자료는(시행규칙 [별표 1]의 제1호 및 제2호에 해당되는 시험자료로 제조·수입량 10톤 미만), QSAR를 통해 얻은 결과를 대신 제출할 수 있다.

QSAR 결과로 시험자료를 대체하여 제출할 수 있는 시험항목

분야	시험항목
가. 물리적·화학적 특성에 관한 시험자료	1) 물질의 상태 2) 물용해도 3) 녹는점/어는점 4) 끓는점 5) 증기압 6) 옥탄올/물 분배계수 7) 밀도 8) 입도분석
나. 인체 유해성에 관한 시험자료	1) 급성경구독성 (단, 물리적·화학적 특성이나 용도상으로 주된 노출경로가 흡입으로 판단되는 경우 급성흡입독성) 2) 복귀돌연변이 3) 피부 자극성/부식성 4) 피부 과민성
다. 환경 유해성에 관한 시험자료	1) 어류급성독성 2) 이분해성 3) 물벼룩급성독성

제13조 제5호 '구조가 유사한 화학물질로부터 얻어진 결과를 통하여 사람의 건강이나 환경에 대한 유해성을 판단할 수 있는 화학물질'의 경우에도 시험자료의 제출을 생략할 수 있다. 같은 조 제3호의 경우 '국제적으로 인정된 QSAR 결과'를 제출하면 시험자료를 제출하지 않아도 되는 반면, 제5호의 경우 대신하는 자료로부터 사람의 건강이나 환경에 대한 유해성을 판단할 수 있으면 관련 시험자료를 생략할 수 있다. 따라서 제5호의 규정에 따라 제출자료를 생략하기 위해서는 화학물질의 '구조가 충분히 유사하다'라는 증거와 함께 그 자료가 '충분히 신뢰할 만하다', '사람의 건강이나 환경에 대한 유해성을 판단할 만하다'라는 것을 과학적으로 보여주는 전문적 판단 자료를 작성하여 함께 제출해야 한다. 그 예로 QSAR 자료 활용과 관련하여 통합적 시험전략(intergrated testing strategy) 마련, 구조 및 대사체 유사성(structure and metabolic similarity)을 입증하는 증거 등이 있다.

2.2. 대체시험자료 활용 원칙

가. 방법 및 원칙

대체시험자료 활용을 위해서는 가용할 수 있는 모든 정보를 수집해야 한다. 정보는 많을수록 좋고, 이후에도 수집된 정보에 대한 전문가의 판단과 적용 가능성 여부에 대한 추가 검토가 필요하다.

1) 관련 있는 모든 정보 수집

대체시험자료을 적용하기 위해서는 가능한 많은 자료원으로부터 관련 있는 기존 정보들을 모두 수집해야 한다. 기존 정보의 활용, 스코어링 및 보고 등에 관한 추가적인 사항은 EU REACH Practical guides - How to use alternatives to animal testing to fulfil your information requirements for REACH registration을 참고할 수 있다.

2) 화학물질의 성질을 결정짓는 자료의 종합적 평가

i) 정보의 취합선별(pooling)

동일한 시험물질 및 시험항목에 대해 다양한 연구 자료 중에서도 신뢰도가 낮은 자료가 있다. 하지만 자료를 취합하다 보면 대부분의 연구에서 영향이 나타나는 농도 및 시간대가 비슷한 것을 확인할 수 있는데, 이 경우 특정 시험항목에 대한 결론을 도출하고 정보 필수 요구사항을 충족시키기 위해서는 모든 연구를 종합적으로 활용하여 타당성을 입증해야 한다.

주요 자료로써 충분히 검증되지 않은 연구는 다음과 같다.

- 이상 있는 시험(Problematic tests): 노출 농도의 타당한 예측값을 결정할 수 없고, WoE 접근법의 일부가 아닌 이상 시험 결과는 신중하게 검증되어야 함
- Klimisch 2, 3 & 4 스코어 연구
- 표준 지침서를 준수하지 않은 연구

[예시]

어류 급성독성

어류 급성독성을 다루기 위해서는 다음과 같은 정보들을 고려할 수 있다.

- 단기 노출(예. 24시간)에만 활용 가능한 유효 어류독성 데이터
- 초기 24시간 내에 주요 영향이 관찰되는 96시간 노출시험(예. 자료가 부족하여 신뢰성이 낮을 것으로 판단될 수 있음). 따라서 24시간 노출시험의 독성값을 결과로 사용 가능
- 여러 시점에서 시험을 수행한 72시간 독성 데이터, 시간에 따른 영향 곡선을 통해 96시간 결과값 외삽 가능

시험자료의 신뢰성을 평가하기 위해서는 다음과 같은 평가 기준을 근거로 가중치를 합산할 수 있다. 평가 기준은 크게 다섯 가지로 구성되어 있으며 시험물질의 정보와 특성, 연구 설계에 대한 기술, 문서화된 결과, 연구 설계 및 결과의 타당성에 따라 자료의 신뢰성을 평가할 수 있다.

- 시험물질 정보: 물질 기본정보(물질명, CAS 번호, 구조식 등), 순도, 물질 공급처 정보, 물리화학적 특성 정보 등
- 시험법의 특성: 시험법에 대한 설명, 시스템 특성(예, 시험종, 성별, 체중, 먹이 공급 기간, 세포주, 배지 성분, 대사 능력, 시험 장기, 혈청, 항생제 투여 여부 등)
- 연구 설계에 대한 기술: 노출경로, 농도, 기간, 음/양성대조군 포함 여부, 시험동물 및 반복군 수 등
- 문서화된 결과: 명확한 독성결과 및 시험법 기술 여부, 통계처리 방법의 적절성
- 연구 설계 및 결과의 타당성: 시험물질의 특성과 관련하여 결과를 도출할 수 있는 적절한 연구 설계인가? 신뢰할 수 있는 정량적 연구결과인가?

ii) 상충된 연구결과 처리

가용할 수 있는 여러 가지의 연구 결과가 서로 상충할 때 대체시험법 중 하나인 WoE 접근법을 활용할 수 있다. 각 연구는 시험방법과 데이터의 수준 및 독성결과 검토에 따라 가중치가 부여되고 신뢰도가 결정된다. 이후 다양한 가중치들의 균형에 근거하여 최종 결론을 도출하게 된다. 일반적으로 의사결정에 있어 데이터의 수준이 높은 *in vivo*(read-across 정보) 및 *in vitro* 데이터는 QSAR 또는 내부 *in vitro* 시험방법보다 더 높은 가중치를 가지게 된다.

iii) 전문가 판단

전문가의 판단은 대체시험법 적용을 통한 물질 평가의 과정에 있어서 신뢰성과 타당성 및 적절성을 고려하게 되므로 매우 중요한 부분을 차지하며, 이를 통해 서로 다른 정보들을 통합·비교하여 각 데이터의 가중치를 부여하게 된다.

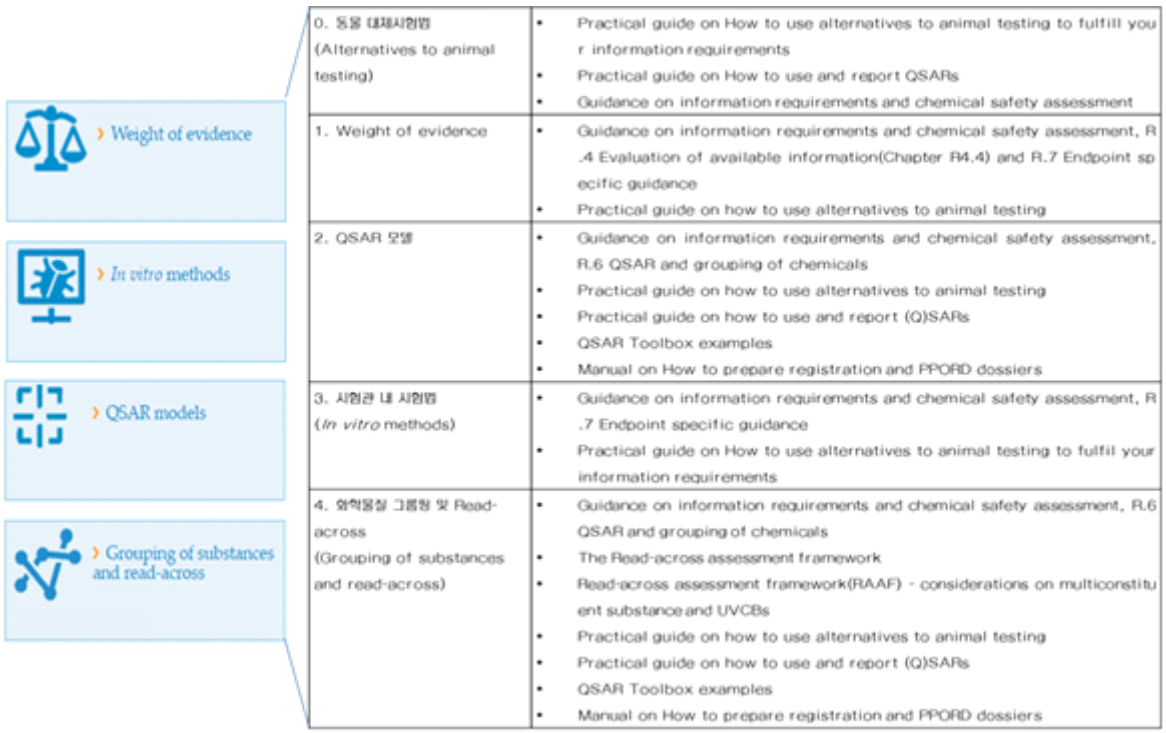
과학적 판단을 제공하는 전문가들은 가용할 수 있는 데이터의 신뢰성과 관련성 및 적절성을 평가하고 화학물질의 성질과 잠재적인 영향에 따른 결론을 도출해야 하므로, 관련 유해성 항목과 시험방법에 대한 전문지식을 반드시 가지고 있어야 할 것이다.

시험 데이터를 이용할 수 없거나 명확한 결론을 도출할 수 없는 경우에는 기타 다른 정보의 활용이나 전문가의 판단을 통해서도 결과를 도출할 수 있다. 전문가 판단을 좀 더 명료하고 이해하기 쉬운 형태로 만들기 위해서는 사용된 모든 정보와 평가과정에서 수행된 절차 및 도출된 결론들을 반드시 기술 문서에 기록하고, 이를 과학적으로도 입증하는 과정이 필요하다.

3. 대체시험자료 활용 방법

3.1. 대체시험자료 종류

대체시험자료는 QSAR와 상관성 방식(Read-across) 및 생체외 시험(*in vitro*) 등으로 구성되어 있으며, 해당 자료의 신뢰도에 따라 가중치(weight)를 부여하여 증거력 방식(WoE)을 적용할 수 있다(그림 2).



0. 동물 대체시험법 (Alternatives to animal testing)	• Practical guide on How to use alternatives to animal testing to fulfill your information requirements • Practical guide on How to use and report QSARs • Guidance on information requirements and chemical safety assessment
1. Weight of evidence	• Guidance on information requirements and chemical safety assessment, R.4 Evaluation of available information(Chapter R4.4) and R.7 Endpoint specific guidance • Practical guide on how to use alternatives to animal testing
2. QSAR 모델	• Guidance on information requirements and chemical safety assessment, R.6 QSAR and grouping of chemicals • Practical guide on how to use alternatives to animal testing • Practical guide on how to use and report (Q)SARs • QSAR Toolbox examples • Manual on How to prepare registration and PPORD dossiers
3. 시험관 내 시험법 (*In vitro* methods)	• Guidance on information requirements and chemical safety assessment, R.7 Endpoint specific guidance • Practical guide on How to use alternatives to animal testing to fulfil your information requirements
4. 화학물질 그룹핑 및 Read-across (Grouping of substances and read-across)	• Guidance on information requirements and chemical safety assessment, R.6 QSAR and grouping of chemicals • The Read-across assessment framework • Read-across assessment framework(RAAF) - considerations on multiconstituent substance and UVCBs • Practical guide on how to use alternatives to animal testing • Practical guide on how to use and report (Q)SARs • QSAR Toolbox examples • Manual on How to prepare registration and PPORD dossiers

[그림 2] WoE로 활용가능한 동물대체시험법(EU REACH 관련 지침서)

가. 증거력 방식(WoE)

1) 과학적 개념

WoE는 개별 정보 또는 한 개의 시험결과만으로 표준 정보 요건을 설명하기에 불충분한 경우, 다양한 자료에서 얻은 정보를 취합하여 적절한 근거를 마련하는 방법을 말한다. 체계적인 절차나 전문가의 판단을 통해 객관적인 방식으로 WoE의 타당성을 입증해야 하며, 데이터의 질, 시험결과의 일관성, 영향의 특성 및 정도, 정보의 관련성 등을 평가하여 이용가능한 정보인지에 따라 가중치를 부여한다.

2) 원칙

이용 가능한 모든 자료를 활용하여 적절한 정보를 제공할 수 있으며 다른 시험결과들을 조합하여 물질 특성에 대한 결론을 도출할 수 있다. 사용된 정보들은 정보 요건을 충족해야 하므로 WoE 접근법을 서술적 요약으로 표현하기도 한다.

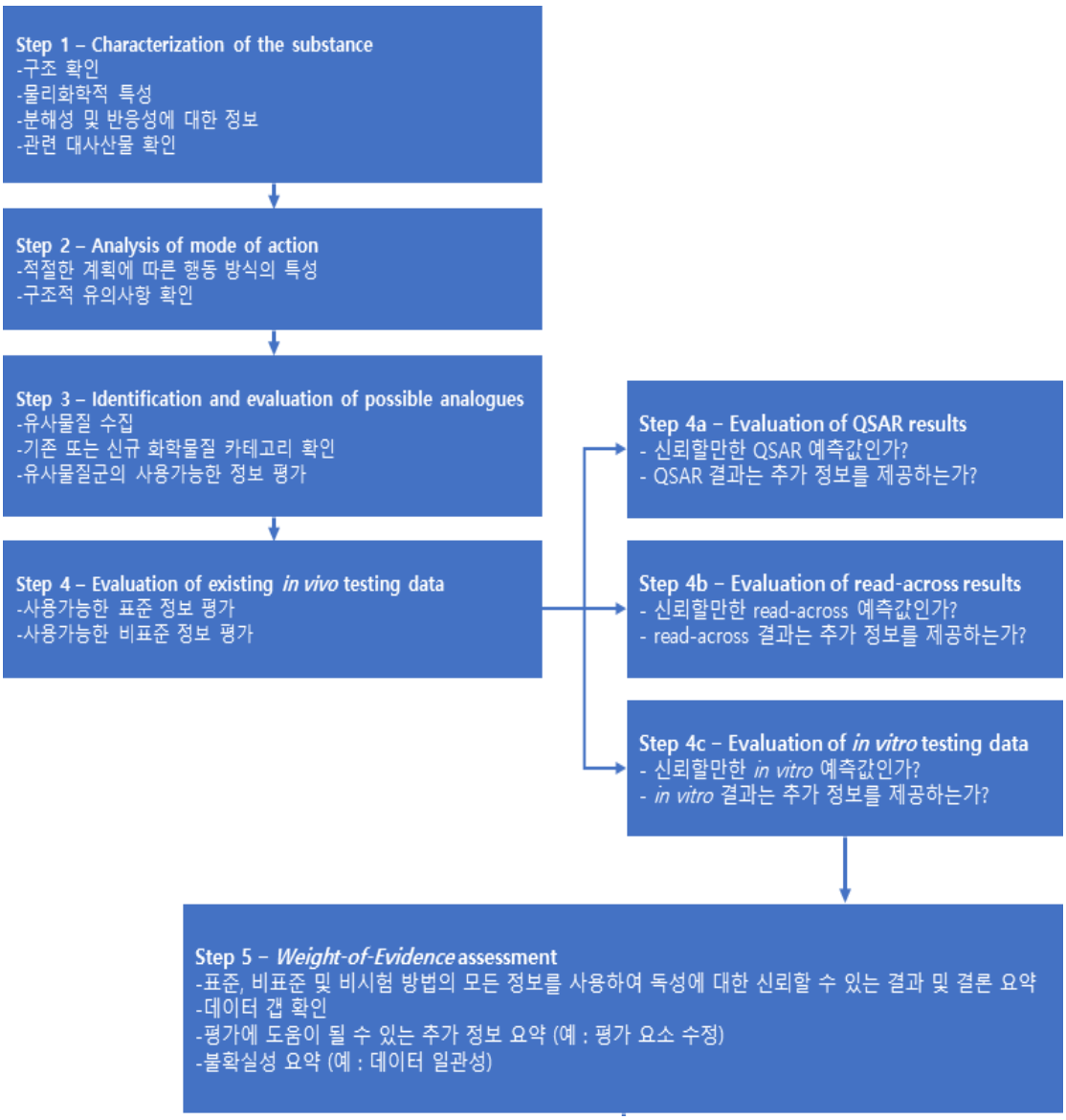
아래에 설명되는 QSAR, read-across, *in vitro* 시험 등은 WoE의 개별 보충자료로 활용될 수 있다.

[WoE 절차]

i) 모든 이용가능한 정보를 수집한다.

ii) 전문가 평가를 통해 타당성을 확인하고, WoE를 이용한 결론을 내린다.

iii) 관련 정보를 기록하고 보고한다.



[Phenolic benzotriazole의 환경 중 잔류성을 평가하기 위한 WoE 접근 방법]

4,6-Substituted phenolic benzotriazole의 환경 중 잔류성 평가 사례

(접근방법)

본 WoE 평가방법은 다섯 가지의 요소를 기반으로 하며, 첫 번째는 잔류성에 대한 스크리닝 연구와 QSAR 예측이다. 두 번째부터 네 번째까지는 3개의 독립적인 모의 실험연구에 대한 평가로, 하나는 실험실 조건에서 예시의 물질에 대한 분해 시험이고, 하나는 분해 거동에 대한 현장 연구, 다른 하나는 유사한 물질에 대한 분해 연구이다. 이러한 모의실험은 항상 결함을 가지고 있기 때문에 그 자체 연구결과만으로 분해성 반감기를 결정하는데 사용할 수는 없다.

WoE 접근방법의 마지막 다섯 번째 요소는 실제 환경에 대한 관찰 사례이다. 이처럼 전체적인 WoE 접근법은 평가가 순전히 질적인 방식으로 수행된다는 것을 의미하는 서술적 요약의 접근 방식으로 이해할 수 있다.

(평가방법)

Phenolic benzotriazole는 페놀의 4,6- 위치에 치환기가 있는 2-(2H-Benzotriazole-2-yl) phenols로 정의되며, 주로 서로 다른 거대한 알킬 또는 알킬아릴 치환기가 있다. 본 WoE 접근방법에서 이를 충족하는 물질들 중 UV-320, UV-327, UV-328, UV-350 등 4개 물질을 평가에 활용하였다.

● 이분해성에 대한 실험실 연구와 QSAR 예측

4개 물질 중 3개 물질은 이분해성 시험(UV-320 및 UV-327는 OECD 301C, UV-328는 OECD 301B)이 이용 가능하였다. 모든 물질이 전혀 BOD를 가지지 않는 등 28일 이내 무기화되지 않았다. 4개 Phenolic benzotriazole 모두 EPISUITE 모델을 이용한 QSAR 계산법을 사용하여 분해도를 계산하였다. 각 모델값에 대해 UV-320, UV-327 및 UV-328는 충족하였으며 UV-350은 경계선에 있었으나(아래 표 참조), 페놀성 벤조트리아졸 그룹 자체는 생분해가 어려운 것으로 알려져 있어서 UV-350 또한 쉽게 생분해되지 않는 것으로 평가되었다.

	UV-320	UV-327	UV-328	UV-350
BIOWIN 2	0.016 (does not biodegrade fast)	0.0013 (does not biodegrade fast)	0.0108 (does not biodegrade fast)	0.1329 (does not biodegrade fast)
BIOWIN 6	0.0091 (not readily biodegradable)	0.0024 (not readily biodegradable)	0.0096 (not readily biodegradable)	0.012 (not readily biodegradable)
BIOWIN 3	1.1165 (months)	1.8338 (>1 month)	2.0546 (months)	2.2538 (weeks–months)
Overall conclusion acc. to QSAR criteria of ECHA guidance R.11[a]	Screening criterion for persistence met	Screening criterion for persistence met	Screening criterion for persistence met	Screening criterion for persistence not met, borderline case

[a] (BIOWIN2 <0.5 AND BIOWIN3 <2.2) OR (BIOWIN6 <0.5 AND BIOWIN 3 <2.2)

● 모의실험 결과

UV-320, UV 327, UV-328, UV-350은 매우 유사한 구조를 가지는 phenolic benzotriazole이다. 모의실험에서 하이드록실 그룹의 ortho 위치에 있는 사이드체인이 단계적으로 분해되는 공통적인 분해 경로를 보여준다. 따라서 각각의 물질에 대한 결과를 일반화하여 read-across 평가에 사용하였다.

① 실험실 내 분해연구

UV-327, UV-328 및 UV-350의 경우, 실험실 모의실험에서 토양 및 퇴적물에 결합하고, DT_{50} 값이 100일 이상임을 나타낸다.

② 현장 분해연구

UV-327 및 UV-328에 대한 현장 모의실험에서는 DT_{50} 값이 151~218일로 추정된다.

③ 유사물질에 대한 분해연구

이와 밀접하게 관련된 유사물질인 EC 407-000-3(P)의 경우 호기성 및 혐기성 모의실험을 수행하였다. 이 물질의 주요 대사산물인 M1는 4개의 Phenolic benzotriazole과 구조적으로 유사하기 때문에 분해성 read-across 자료로 사용하였으며, 그 결과 UV-320, UV-327, UV-328 및 UV-350이 더 낮은 속도로 분해되며 지속성은 더 높을 것으로 예상할 수 있었다. 시스템의 유기물 함량에 따라 호기성 조건 하에서 퇴적물의 DT_{50} 값은 M1의 경우 약 248일, 혐기성 조건 하에서는 최소 238일로 확인된다. 따라서 M1은 환경에서 잔류성이 있음을 간주할 수 있다.

● 실제 환경의 분해성 연구 사례

서로 다른 목적과 방법이 사용된 실제 환경에서 분해 연구 사례를 비교한 결과 Phenolic benzotriazole의 생산기간 동안과 생산기간 후 12~25년이 경과한 시점의 오염농도가 유사한 수준으로 분해가 매우 낮은 것으로 확인되었다.

● 불확실성 평가

각각의 정보원(source) 그 자체로는 직접적인 평가자료로 활용하기에 제한적이며, 불확실성을 지니고 있다(페놀성 벤조트리아졸이 환경에서 잔류성이 있다는 결론을 내리기는 불가능함). 하지만 WoE 접근법을 통해 사용된 각각의 정보들을 서로 결합하면 각각의 결점은 없어지고 불확실성이 낮아져 평가에 활용 가능하다.

(결론)

위의 내용들을 정리하면, 다른 source로부터 이용 가능한 정보들은 Phenolic benzotriazole이 환경에서 잔류성을 지닌다는 것을 나타내고 있다. 더불어 UV-327 및 UV-328에 대한 모니터링 연구뿐만 아니라 유사물질에 대한 모의실험에서 담수 퇴적물 내 물질의 반감기가 180일보다 길다는 것이 확인되었으며, 이는 매우 강한 잔류성을 가지는 것으로 볼 수 있는 활용 가능한 결과이다. 개별적 정보들을 종합적으로 볼 때 매우 잔류성이 강한 물질이라 결론을 내릴 수 있다.

나. QSAR

1) 과학적 개념

구조 활성관계 예측 프로그램(QSAR)은 이론적인 모델링으로서 화학물질의 구조식을 분석하여 물리화학적, 생물학적(유해성항목), 환경 거동 특성을 정성적 또는 정량적으로 예측하는 접근법이다. QSAR는 모의시험법(*in silico* approach)으로도 명명되며, 불필요한 동물시험 등을 대체할 수 있다. 그러나 예측모형으로 도출한 결과는 유효성을 고려해야 하며, 특정 조건에서만 사용될 수 있다는 것을 고려해야 한다.

2) 원칙

i) QSAR 예측 결과를 적용할 수 있는 일반적인 원칙은 다음과 같다.

- 화학물질이 QSAR 모델의 적용가능한 영역에 있어야 한다.
- 예측 결과가 과학적으로 유효한 모델을 사용하여 도출되어야 한다.
- 예측 결과가 분류 및 표시, 위해성 평가 등에 적절해야 한다.
- 적용 방법에 대한 정보가 문서로 제공되어야 한다.

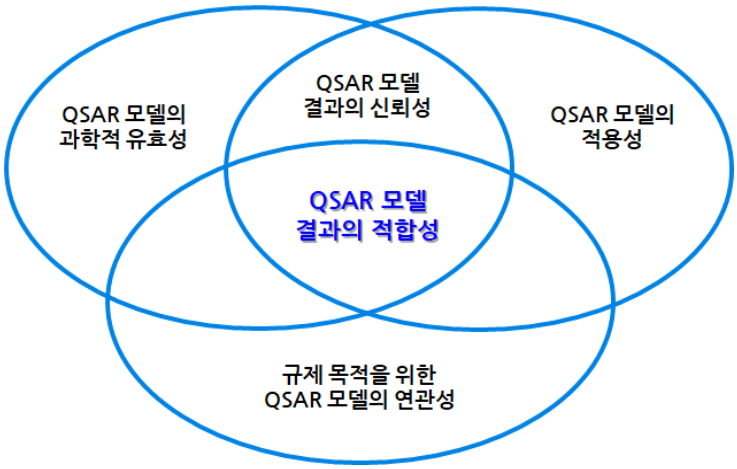
ii) 과학적으로 유효한 QSAR 모델은 다음 원칙에 따라 평가된다.

- 명확한 유해성항목
- 명확한 알고리즘
- 명확한 적용 범위
- 적절한 절차를 거친 적합도(goodness-of-fit) 검증 등 충분한 통계적 특성으로 기술
- 매커니즘적 해석(가능한 경우)

[활용방법]

QSAR 예측 모델을 적용하기 위해서는 신뢰성(예, 적용가능한 영역에 있는 유효한 QSAR 모델로부터 도출)과 규제적 의사결정을 위한 연관성을 고려해야 한다. 분류 및 표시, 위해성 평가를 위한 예측의 적합성은 유해성 항목에 따라 달라지며, 규제적 의사결정을 위해서는 예측의 적합성을 평가할 수 있는 추가적인 정보가 필요하다.

이에 따라 유효성(“모델의 과학적 유효성에 대한 OECD 다섯 가지 원칙을 충족하는가?”)과 적용성(“모델을 대상물질에 적용하는 경우, 예측 결과는 신뢰할 수 있는가?”) 및 연관성(“위해성 평가 또는 분류 및 표시를 위한 정보를 생산해야 하는가?”)에 대한 각각의 평가가 필요할 것이다.



[그림 3] QSAR 모델의 적합성 판단 기준

[유기화학물질에 대한 QSAR 방법 적용]

본 예시는 화학 카테고리(read-across 활용안내서에서는 '구분'으로 표현하고 있다.) 개발에 QSAR 방법을 사용할 수 있는 몇 가지 다양한 방법을 요약하고 설명하는 것을 목적으로 한다. QSAR 방법이라는 용어는 유사체 검색 방법, 탐색 데이터 분석, 모델 개발을 위한 통계 방법, SAR 및 QSAR에 기반한 기존 계산 예측 방법을 통합하기 위해 가장 광범위한 의미로 해석될 수 있다.

1. 존재하는 category의 category 구성체(member) 평가

새로운 물질이 유사한 방식으로 평가되어야 하는 만큼 category의 기존 member와 충분히 유사한지 확인해야 한다. 이를 위해서는 QSAR 모델링 접근법을 적용하기 위해 먼저 category member를 화학적 측면으로 특성화해야 한다. 본 연구에서는 HMWPE(2)의 SIAM category를 조사하였다. 이 category의 7개 member는 다성분 물질(multi-component substances)이며, 따라서 모델링을 목적으로 특성을 지정하는 것이 복잡하다. 이 경우에는 아래와 같은 사항이 고려되어야 한다.

a) 혼합물에서 가능한 모든 구성요소(알려져 있거나 의심되는지, 그리고 성분은 대략 동등한 성분 비율을 나타내는)
b) 혼합물에서 우세한 성분
c) 구성요소의 사슬 길이와 가지를 반영한 대표 구조

또한 물질이 category에 속하며, 해당 category의 합리적인 member인지에 대한 질문도 있기 때문에 category의 경계를 나타내는 선택 구조가 추가 고려사항에 포함될 수 있다.

2. category 가설과 category 정의 확인

초기 가설은 예를 들어 각 화학물질인 프탈레이트 에스테르가 프탈레이트 일부분과 동일한 특성 및 추세를 보여 준다는 것이다. 이러한 일반화에 명백히 결함이 있을 수 있지만 다양한 방법을 사용하여 도출된 독성결과들로 적합한 적용영역을 정의할 수 있는 점을 찾기 위한 시작점이 되었다.

2.1 유사체 탐색에 대한 전략

새로운 category를 개발하거나 기존 category를 확장할 때 read-across를 수행하는 초기 단계는 실험 데이터를 사용할 수 있는 유사 항목을 검색한다.

2.2 탐색적 분석: 유사성, 차이점 및 특이점 식별

실험 데이터가 없는 경우 다양한 탐색 데이터 분석 방법이 화학물질의 유사데이터에 적용되었다. 이러한 방법은 화학물질 간의 관계를 시각화하여 유사성을 탐색하고 가능한 이상값을 식별하는 편리한 수단을 제공한다. 이 뿐만 아니라 탐색적 데이터 분석을 위한 일반적인 통계법인 PCA 주성분 분석을 이용한다. 이것은 데이터 세트의 화학적 공간을 시각화하는데 사용할 수 있다.

2.3 탐색적 분석: 추정된 독성결과의 매트릭스 구축

실험데이터가 없거나 부족한 경우 기존 QSAR모델을 사용하여 다음을 수행할 수 있다. a) 공통적인 유해성이 있는 화학물질을 식별한다, b) 유해성에 대한 잠재성 경향을 예측한다, c) 추세에서 가능한 중단점을 식별하므로 가능한 하위 category를 확인한다. 이 접근법을 설명하기 위해 유사체 데이터 세트는 여러 독성결과에 대한 예측을 포함하도록 확장되었다.

2.4 데이터 수집- 추정 값으로 데이터 갭 채우기

신뢰 가능한 실험 데이터가 없는 경우 유효한 QSAR 모델을 사용하여 데이터 갭을 채울 수 있다. 사용된 모델은 QSAR 검증을 위해 OECD 원칙에 따라 검증되어야 한다. 가능한 한 QSAR방법에 의해 설정된 예측 및 추세는 실험 데이터와 비교하여 검증되어야 한다. 실험 데이터는 최소한 일부 category member에 대해 사용가능해야 하므로 이 접근법에 대해 타당해야 한다.

3. 실험데이터의 적합성 평가

데이터 타당성은 Klimisch, OECD 등 신뢰성 평가방법 등을 참조하여 평가할 수 있다. 신뢰할 수 있는 (Q)SAR 예측은 추가 정보를 제공하여 데이터 적절성 평가에 기여할 수 있으며, QSAR 추정치는 실험 데이터에 기초한 의사결정에 있어서 WoE를 추가할 수 있다.

4. Read-across의 적합성을 평가

Read-across를 적용하는데 어려움 중 하나는 유사물질과 타겟 물질 사이의 작은 구조적인 차이가 read-across를 무효화할 수 있다는 불확실성이다. 동일한 category 또는 하위 category 내에서 read-across 작업이 수행될 수 있도록 기계적 고려사항을 염두에 두고 category를 개발해야 한다. 과학적인 과제는 이러한 하위 category를 정의하기 위한 적절한 구조 규칙 또는 물리적·화학적 차단 값을 정의하는 것이다.

5. 결론

- Read-across 적용을 위한 유사체 확인이 가능한 많은 검색 엔진들이 있으며, 이러한 검색 엔진들은 유사성을 가진 다른 유사체들을 제공한다.
- PCA 및 클러스터링과 같은 데이터 탐색 도구는 화합물 세트의 화학적 영역을 시각화하여 명백한 '유사' 화합물 그룹을 찾는데 유용하다. 이러한 접근법은 화학물질의 시작 데이터와 화학물질에 대한 서로 다른 매개변수(기하학적, 위상학적, 구조적, 물리 화학적, 전자적 설명자 등)를 계산하거나 구조적 지문을 사용하여 특성을 분석한다.
- 기존 QSAR의 예측을 사용하면 화학물질 그룹 내 추세를 탐색하거나 데이터의 적합성을 평가하는데 도움이 될 수 있다.
- 마지막으로, QSAR 방법은 category 형성에 유용한 보조 도구로 활용할 수 있다.

다. 상관성 방식(Read-across)

1) 과학적 개념

Read-across는 대상 물질의 유해성 시험의 정보를 다른 물질의 동일한 유해성 시험의 정보를 사용하여 예측하는 기술이다. 물질의 물리화학적, 독성, 생태독성 정보는 화학물질의 구조식에 따라 유사한 양상을 나타내며, 이러한 물질을 '하나의 그룹'으로 고려한다. read-across에서 '아날로그(유사성에 기반한) 접근법'은 구조적으로 유사한 물질의 수가 적은 경우 적용할 수 있다. 명확한 구조적 유사성으로 같은 그룹에 속해 있으면서 물질들 간에 차이가 존재하는 물질들에 read-across가 적용되는 경우 'category에 기반한 접근법'을 사용한다.

2) 원칙

확보한 정보에서 유사 물질에 대한 기존 시험 결과 유무 등을 확인하기 위해 등록 대상물질의 특성을 예측하는데 있어 '유사성' 규칙에 따라 read-across를 적용할 수 있는지 고려해야 한다. 유사 물질은 국제적인 물질 평가(OECD HPV 카테고리 접근법) 또는 전문가 툴(OECD QSAR Toolbox)을 통해 확인할 수 있다.

[활용방법]

대상 물질의 구조적 유사성을 평가해서 구조적 차이점이 특정 유해성 시험결과에 미치는 영향을 고려해야 한다. 물리화학적 특성을 확인하여 read-across에 적용할 수 있는 과학적 근거를 마련할 수 있으며, 그룹핑/카테고리 정의는 물질의 특성/작용에 대한 유사성을 설명할 수 있어야 한다.

적절한 read-across 접근법을 선정하기 위해서는 과학적인 근거에 기반한 시나리오를 확인해야 한다. 시나리오는 물질 특성에 대한 read-across 예측 기법을 평가하기 위해 개발되었으며, 이때 '물질 특성'이라 함은 독성학적 영향(인체 건강), 생태학적 영향 및 환경 거동에 대한 특성을 의미한다. 이에 따라 read-across 접근법은 '아날로그 접근법'과 '카테고리 접근법' 두 가지로 구분할 수 있다.

[2-Ethylbutyric acid(2-EBA)의 90일 반복투여독성 평가를 위한 Read-across 활용 방법]

2-Ethylbutyric acid(2-EBA)의 90일 반복투여독성 예측 사례

(접근방법)

2-Ethylbutyric acid(2-EBA) 등록을 위해 필요한 90일 경구독성시험 결과가 없는 상황으로 9개 유사물질의 아만성 독성연구결과를 활용하여 90일 경구독성시험 결과를 예측하고자 하였다. 예측한 결과에 대한 입증을 위해 음성대조군 1개를 사용하여 read-across 접근법을 활용하였다.

- 9개의 유사물질: 2-PHP(2-Propylheptanoic acid), 2-EHP(2-Ethylheptanoic acid), 2-PHA(2-Propylhexanoic acid), 2-EHA(2-Ethylhexoic acid), VPA(2-Propylvaleric acid), 2-EPA(2-Ethylpentanoic acid), 2-MHA(2-Methylhexanoic acid), 2-MPA(2-Methylpentanoic acid), 2-MBA(2-Methylbutyric Acid)
- 1개의 음성대조물질: PVA(Pivalic acid)

(평가방법)

In vivo, in vitro, 세포독성, QIVIVE 자료 활용을 통한 예측

• *In vivo*

10개 물질 중 3물질(2-EHA, VPA, PVA)은 경구 및 복막 내 반복투여 독성시험 결과가 존재하였다.

두 유사 source물질(2-EHA, VPA)은 *in vivo* 경구 반복투여독성시험, 2-Ethylhexanoic acid (2-EHA)는 아만성 반복투여독성시험 결과가 있었으며, 시험결과 상대적으로 간 무게 및 간비대 증상이 관찰되었다(이는, 간세포에 영향을 미치는 것을 의미). 또한, Valproic acid (VPA)의 아급성 연구 결과, 간 미세수포성 지방증이 확인되었다.

Pivalic acid (PVA)는 데이터의 적용성 판단을 위해 필요한 물질로서 음성대조군으로서 시험되었다. PVA는 아급성 연구에서 가장 높은 시험 용량을 투여할 때까지 어떠한 간 독성도 유발하지 않았다.

• *In vitro*

in vivo 테스트를 바탕으로 2-EBA가 지방증을 우려하는 간 독성물질임을 read-across 가설로 세울 수 있다. 이러한 read-across를 이용한 평가에서 AOP(adverse outcome pathway)는 주요 독성 영향으로 간 지방증에 대해 알려져 있고, 간 지방증의 생성에 대

해 설명이 가능하다. AOP 경로에 지방증을 유발하는 약 50개의 신호 경로가 요약되어 있다. 9개의 source물질에 대한 확인을 위해 *in vitro* 모델로 시험하는 MIE(molecular initiation events)와 KE(key event)을 사용하였다.

① MIE(molecular initiation events)

높은 처리량 모델인 CALUX 및 GFP 리포터 분석을 통해 AOP 경로에 존재하는 6개의 MIE를 측정하였다. 그 결과, 체인길이가 증가함에 따라 지방증과 관련한 활성 MIE들이 증가하였다. 또한, 2-EBA는 PPAR-α 지방증과 관련하여 하나의 MIE를 활성화하였고, 이는 지방질 축적(KE)에 대한 경로를 배제할 수 없음을 알 수 있다.

② KE(key event)

AOP에서 KE는 지방질 축적이다. 이는 실제 *in vivo*에서의 독성발현과 매우 근접하며 read-across 가설을 확인하는데 매우 중요하다. 3개의 간 모델을 이용하여 간 지방증에 대한 KE인 세포내 트리글리세라이드 축적을 측정하였다. 단일 또는 반복 노출 후 주로 긴 체인의 유사체에서 지방질 축적이 관찰되었고, 짧은 체인의 유사체들은 비활성화인 체로 남아있었다.

⇒ 간지방증에 대한 *in vivo* 데이터를 가진 두 화합물들(2-EHA, VPA)은 지방질 축적을 유발한 반면, *in vitro* 실험에서 2-EBA의 HepG2 and HepaRG 세포에서 가장 높은 시험용량까지 비활성적이었다. 음성대조군 Pivalic acid (PVA)은 *in vivo*에서 간 독성이 없었고, *in vitro* 실험에서도 가장 높은 용량을 투여할 때까지 비활성적이었다.

• 세포독성

일반적인 생물학적 경로와 세포 기능의 변화(글루타티온(GSH) 고갈, 미토콘드리아 막전위의 붕괴, 과산화물 형성 및 인지질화 등과 같은 HepG2 세포의 기본적인 생물학적 과정)에 대해 세포독성(간 그리고 신장 세포에서)과 관련한 시험을 통해 9개의 source물질 사이의 차이점을 확인하려 했지만 차이점을 확인할 수 없었다. 전반적으로 사이드체인의 길이가 줄어들면 세포독성이 낮아지는 경향은 확인되었으나, AOP 경로 또는 일반적인 생물학의 변화에 대한 MIE는 다른 시험결과에서 나타나지 않는다.

• QIVIVE

Read-across의 목적은 아만성 *in vivo* 동물 연구를 통해 누락된 데이터 갭을 채우는 것으로 QIVIVE(Quantitative *in vitro* to *in vivo* extrapolation)에는 PBPK 모델이 사용되었다.

쥐의 PBPK모델은 *in vivo* 데이터를 기반으로 입증되었으며, 혈장 및 해당 장기의 농도를 계산하는데 사용되었고, *in vitro* 시험에 대한 농도 선택을 유도하였다.

인간의 PBPK 모델은 물리화학적 특징과 *in vitro* 승인 데이터(예. 혈장 단백질 결합(ppb)과 실질적인 간세포 승인)를 기반으로 모든 read-across 물질에 대해 입증하였고, VPA에 대한 인간의 *in vivo* 약동학 데이터는 인간에 대해 관찰된 혈장 농도 데이터를 기반으로 예측성능을 확인하고, 증명하였다.

이 개념 증명을 기반으로 IVIVE-PBPK 모델은 모든 유사물질에 대해 *in vitro*에서 *in vivo* 외삽에 사용되었다.

(결론)

세 물질이 간 지방증을 유도하는지 여부를 *in vivo* 데이터를 통해 예측하였다. 2-EBA는 모든 실험에서 *in vivo*의 동물 데이터를 가진 두 유사체 2-VPA와 2-EHA보다 간 독성이 덜하였다. 그리고 *in vitro* 시험에서 가장 높은 용량까지 간 지방증을 유도하지 않음을 나타낸다는 점을 확인하였다. source물질들의 신장 모델들은 세포독성이 낮았고, 세포독성에서 물질간의 차이점이 나타나지 않았다. 또한, 미토콘드리아 막 전위 또는 일반적인 생물학적 과정의 변화를 측정하는 독성시험결과에서 체인길이가 길수록 더 많은 활성도의 추세를 유지하였다.

위해성 평가를 위한 경구 용량을 도출하기 위해 시험관내 외삽법(QIVIVE)을 적용하였다. 2-EBA의 경구 용량 중 가장 낮은 *in vitro* 독성시험결과의 백분위 값을 사용한 QIVIVE는 랫드의 경구용량이 730~948.6 mg/kg bw/d로 확인되었으며, 이는 아만성 독성연구의 데이터 갭을 채우는데 사용할 수 있었다. 더불어 QIVIVE는 인간 경구용량 값을 직접적으로 결정하는데 사용되었고, 이는 243~245.7 g/kg bw/d로 추정된다.

라. 생체외(*in vitro*) 시험

1) 과학적 개념

생체외 시험(*in vitro*)은 시험관, 페트리 접시 등 생물 체외에서 시험조건을 조절하여 결과를 도출하는 방식이다. 유럽동물대체실험검증센터(ECVAM)의 사전검정기준 등 국제적으로 정해진 시험수행기준에 따라 *in vitro* 방식으로 도출한 결과는 물질의 특성을 확인하고 물질의 작용 방식을 이해하는데 유용하다.

2) 원칙

생체외 시험(*in vitro*)은 생체내 시험(*in vivo*)을 고려하기 전에 물질의 특성에 대한 정보를 생성하는데 적합하며, 사전 검토 단계에서 WoE 접근법 내에서 고려된다. REACH 부속서에서는 피부 및 눈 자극성, 피부 과민성 등에 대한 대체시험법으로 *in vitro* 시험을 명시하고 있다. 하지만 *in vitro* 시험이 국제표준에 따라 수행되지 않은 경우, 이용 가능한 모든 정보를 취합하여 WoE를 적용하고 정보 요건에 충족하는 등록서류를 제출해야 한다.

3.2. 적용 방법

가중치를 부여하여 WoE 자료로 활용할 수 있는 QSAR 및 read-across에 대한 화평법 적용 조건은 다음과 같다.

가. QSAR

화학물질의 등록 및 평가를 위한 시험 자료의 경우, 국제적인 시험지침(예. OECD guidelines 등)과 GLP 규정 등을 준수하여 수행된 시험에 대해 국제적인 규제 목적의 근거자료로 활용될 수 있다. QSAR 자료의 경우도 기본적인 원칙을 충족하는 프로그램으로서 등록자가 충분한 지식을 활용하여 확보한 결과의 경우, 시험 자료를 대신하여 등록 자료로 제출할 수 있다. 규제 목적 활용을 위한 QSAR 결과의 적용조건은 다음과 같은 4가지 원칙으로 설명될 수 있다.

1) 과학적 유효성(scientific validity)이 확립된 모델에서의 예측

QSAR 결과가 실험 데이터를 대체할 수 있고 화학물질 법적 규제를 위한 평가에 이용되기 위해서는 타당성에 대한 검증이 필요하다. 타당성을 검증하기 위해 OECD는 QSAR에 대한 5가지 원칙을 제시하였으며, 규제적 목적을 위해 과학적으로 검증된 5가지 원칙은 다음과 같다.

i) 정의된 평가항목(a defined endpoint)을 갖는다.

원칙의 목적은 주어진 모델에 의해 예측되는 평가항목의 투명성을 보장하는 것이다. QSAR 모델은 정의된 평가항목과 관련되어 있어야 하며, 물리화학적 특성, 인체건강, 생태학적 독성 및 환경거동 매개변수 등의 평가항목들은 측정되고 모델링 될 수 있다. 주어진 평가항목은 다양한 실험 프로토콜과 다양한 실험조건에 따라 결정될 수 있다.

ii) 명확한 알고리즘(an unambiguous algorithm)으로 기술된다.

원칙의 목적은 모델 알고리즘을 기술하는데 있어 투명성을 보장하는 것이며, 이에 따라 QSAR 모델은 명확한 알고리즘 형태로 표현되어야 한다.

iii) 정의된 적용 영역(a defined domain of applicability)을 갖는다.

QSAR 모델은 정의된 적용 가능한 영역과 관련 있어야 한다. 적용 가능한 영역은 화학구조, 물리화학적 특성 및 작용 매커니즘을 통해 예측을 수행할 때 불가피하게 갖게 되는 한계성을 보완한다.

iv) 충분한 통계학적 특성(statistical characterization)으로 기술된다.

QSAR 모델은 적합도(goodness of fitness), 견고성(robustness) 및 예측도(predictability)를 적절히 측정해야 한다. 이 원칙은 다음과 같은 두 가지 정보를 요구한다.

- 훈련 세트(training set)를 이용하여 결정되는 모델의 내적 성능 적합도, 견고성
- 적합한 테스트 세트(test set)를 이용하여 결정되는 모델의 예측도

v) 매커니즘 해석(a mechanistic interpretation)을 갖는다.

원칙의 목적은 모델에 사용하는 기술자(descriptor)와 예측되는 평가항목 간 매커니즘 해석과 이를 문서화하는 것이다. 가능하다면 QSAR는 매커니즘 해석이 가능해야 하며, 해석이 가능한 경우 원칙 i)~iv)를 기반으로 확인된 모델의 신뢰도를 높일 수 있다.

2) QSAR의 적용 범위(applicable domain) 이내에서의 예측

시험자료를 대체하여 QSAR 결과를 제출하려는 등록대상 화학물질은 특정 QSAR 모델의 적용 범위에 포함되어 있어야 한다. 적용 범위와 관련하여 다양한 예가 있지만, 다음과 같은 사항들을 사전에 검토해야 한다.

i) 화학적 범위(chemical domain): 구조(작용기 또는 작용기 배열)와 물리화학적 성질이 같은 범위에 있는가?
ii) 생물학적/독성학적/기계론적 범위(biological/toxicological/mechanistic domain): 동일한 작용기작을 갖거나 활성영역을 갖는가?
iii) 대사 범위(metabolic domain): 생체 내 전환 또는 대사 형태가 동일한가?

3) 분류・표시 및 위해성평가 등에 적합한 결과

규제적 목적으로서의 QSAR의 적절성은 화학물질에 대한 모델의 유효성(validity)과 적용성(applicability) 및 규제를 위한 모델의 연관성(relevance)를 다룬다. 유효성과 적용성은 QSAR의 신뢰성(reliability)을 결정한다. 관련성과 신뢰성을 통해 QSAR 결과가 적합한 결과로 문서화되는 경우, 해당 결과 자체를 규제 목적으로 활용할 수 있다. 실제 QSAR 결과는 일부 측면에서 불확실성이 존재할 수 있지만, 정보 부족으로 인한 불확실성은 추가 정보수집을 통해 보완할 수 있다. QSAR에 적용할 수 있는 영역은 다음과 같다.

i) 우선순위 결정 절차에 이용할 정보 제공
ii) 실험 또는 시험 전략에 대한 실험설계 안내
iii) 기존 시험 데이터의 평가 개선

iv) 매커니즘 정보 제공
v) 유해성 및 위해성 평가에 필요한 data gap 보완
vi) 분류 및 표시에 필요한 data gap 보완
vii) PBT 또는 vPvB 평가에 필요한 data gap 보완

4) 적절하고 신뢰성 있는 문서 정보의 제공

QSAR 결과는 사용한 프로그램의 특성 등과 같은 모델 자체에 대한 내용과 해당 모델을 통해 얻는 자료를 적절한 서식을 통해 문서화하는 것이 중요하다. 현재 OECD와 유럽 등에서 QSAR 모델에 대한 QMFR(QSAR Modeling Reporting Format)과 예측 결과를 기술하고 평가하는 QPRF(QSAR Prediction Reporting Format)이 활용되고 있다. QMRF는 OECD 검증원칙에 따라 모델에 대한 주요 정보를 보고하는 것으로 검증(validation)과 관련이 있으며, QPRF는 모델로 수행한 화학물질의 예측을 기술하고 평가하는 것과 관련이 있다.

나. Read-across

Read-across를 활용할 경우 원칙적으로 다음 조건을 모두 충족해야 한다. 화평법 시행령 제13 제5호에 따르면 구조가 유사한 화학물질로부터 얻어진 결과를 통하여 사람의 건강이나 환경에 대한 유해성을 판단할 수 있는 경우에 해당 시험자료 제출을 생략할 수 있도록 규정되어 있다. 즉, read-across에 따라 제출되는 자료가 이 규정에 적합하다는 판단을 할 수 없는 경우에는 수용될 수 없을 것이며, 충분히 보수적인 입장에서 수용 가능성을 검토하고, 유해성평가에 활용하게 될 것이다. 따라서 제출자는 read-across를 어떻게 도출하였고, 그것이 왜 의도하는 목적에 적합한지에 대한 판단을 설명할 수 있어야 한다.

1) 적용 조건

i) 대체자료는 분류 및 표시, 위해성 평가의 목적에 적합해야 한다.

참조물질의 read-across 자료가 주요 자료로 사용될 경우, 등록물질에 대해 화평법에서 정하는 적절한 분류 및 표시를 결정할 수 있는 정도로 해당 자료는 적합하고 신뢰도가 높은 견고한 자료이어야 한다. 마찬가지로, 화평법에 규정한 위해성평가나 위해성자료 작성에 사용될 수 있을 정도로 신뢰도가 높은 '용량 기술자(dose descriptor)'를 제공해야 한다.

- 위해성자료 작성 대상일 경우 read-across에서 확보한 용량기술자는 안전성 판단에 활용될 것이다. 이때 사용한 NOAEL 값이 지나치게 독성이 낮게 평가된

것이라면, 해당 용도로 사용할 때 실제로는 위해 우려가 있어 적절한 관리대책이 강구되어야 함에도 실제로는 그렇지 않은 것으로 평가될 수도 있다.

ii) 시험에 사용된 핵심 매개변수(parameter)의 범위는 신뢰도가 높아야 한다.

화평법 제14조 제1항 제5호·제6호 및 시행규칙 [별표 1] 제8호에 따른 시험 방법이나 이에 상응하는 국제적으로 인정되는 시험 방법에서 사용된 핵심 매개변수의 범위는 적합하고 신뢰도가 높아야 한다. 핵심 매개변수의 범위는 참조물질에 대해 취합된 정보의 수준이 최신 시험 방법에 따라 시행한 새로운 시험에서 기대할 수 있는 수준에 부합하는지 확인하는 데에 반드시 필요하다. 즉 대체자료는 그 자체로 충분히 신뢰성이 높고, 관련 규정을 만족하는 것이어야 한다.

iii) 국제적으로 인정받는 시험 방법에 준한 매개변수 및 노출 기간을 다루어야 한다.

관련된 매개변수가 노출이라면 ii)에서 언급된 국제적으로 인정되는 시험 방법에 준하거나 그 이상의 노출 기간을 다루고 있어야 한다. 예를 들어 참조물질의 아만성(90일) 반복투여독성 자료를 활용하여 등록물질의 아급성(28일) 반복투여독성 자료를 read-across 자료로 다룰 수 있으나, 반대의 경우는 적용할 수 없다.

- 현재 소량 화학물질에 대해서도 용도나 예측결과에 대하여 시험자료 제출이 요구되는 경우가 있다. 이 경우에도 참조물질 시험자료의 신뢰성이나 적합성이 모두 i) 또는 ii)를 모두 만족해야 하는 것은 기본적인 사항이다. 다만 제조·수입량이 적고 실제 노출가능성도 적어 사례별 접근방식이 필요한 경우, 제출자가 위의 조건과 연계하여 전문적 설명을 추가하는 것이 필요할 것이다.

iv) 적용된 방법은 적절하고 신뢰성 있는 문서로 제공되어야 한다.

Read-across의 적합성과 과학적 정당성을 독립적으로 평가하기에 충분한 자료를 문서화하여 제출해야 한다. Read-across를 적절히 문서화하기 위해서는 다음 사항을 중심적으로 고려해야 한다.

- Read-across의 가설
- 상기 가설에 대한 가설입증(justification)
- Read-across에 포함된 모든 물질 목록
- Read-across에 포함된 모든 물질의 상세한 동질성(식별) 정보
- Read-across를 적용한 시험항목 목록
- 데이터의 매트릭스
- 제안한 read-across의 적용성에 대한 결론

2) 가설과 가설 입증

Read-across에 대한 가설을 분명하게 제시하는 것이 중요하다. 참조물질과 등록물질 간의 구조적 유사성과 기타 확인된 유사성(분해산물의 유사성, 작용기작의 유사성 등)을 규정할 수 있는 특징을 묘사하기 위해서는 가설 설정이 필수적이다.

Read-across에 대한 가설은 개별 관련 시험항목에 대한 참조물질의 시험결과로부터 왜 등록물질의 특성을 예측할 수 있는지를 설명해야 한다. 그러므로 가설에서는 반드시 read-across가 적용된 시험항목을 제시해야 한다. Read-across를 다수의 시험항목에 적용할 경우, 등록신청인은 개별 제출항목의 다양한 복합요소(핵심 매개변수, 생물학적 표적 등)를 고려하여 각 항목에 대한 적절한 논증(가설과 가설입증)을 제시해야 한다.

가설입증이란 보유한 자료에 기초하여 등록자가 제시한 read-across 가설에 대해 이를 뒷받침하는 사실을 전문적, 논리적으로 보여주는 것이다. 가설입증에서는 또한 적용과정에서 확인된 단점과 read-across와 관련된 불확실성에 대한 설명도 개략적으로 제시되어야 한다.

따라서 read-across를 적용하는 입장(산업체)에서나 수용여부를 결정하는 입장(국립환경과학원)에서 가장 중요한 부분은 바로 (등록물질 A의 28일 반복투여독성이 참조물질 B와 유사하다는)가설을 세우고, 그 가설을 입증하는 것이라 할 수 있다. 이중 전문성은 가설을 입증하는데 있어 매우 핵심적인 역할을 한다. 가설의 과학적 타당성과 논리성이 부족하거나 가설입증 과정이 합리적이지 못할 경우, 제출된 자료는 수용되지 않을 것이다. 특히 28일 반복투여독성시험과 같이 해당 시험항목이 (위해성에 관한 자료작성에서) 안전성을 확인하거나 분류·표시에 중요한 경우, 유독물질 등 유해화학물질 여부를 판단하는 결정적인 정보일 경우에는 가설과 가설입증에 많은 전문성을 투입해야 할 것이다.

4. 결론

4.1. 기대효과

대체시험자료는 동물실험자료를 대신하여 물질에 대한 유해성 등을 확인할 수 있는 자료로 QSAR, read-across, WoE, *in vitro* 등을 포괄한다.

대체시험자료 활용을 통해 기대할 수 있는 효과는 다음과 같다.

i) 주요자료로 충분히 검증되지 않은 신뢰성이 낮은 연구자료를 활용할 수 있음
ii) 화학물질 성질에 대한 결론을 도출할 수 있음
iii) 상기 i), ii)에 따라 정보의 필수 요구조건을 충족할 수 있음

이는 한 가지 유해성 시험항목을 위해 가용할 수 있는 모든 정보와 서로 다른 데이터 소스의 사용을 최적화하는 방법 중 하나로, 대체시험자료를 타 연구와 결합하여 분석하게 되면 충분한 정보를 제공받을 수 있게 된다.

이때 증거에 기반한 접근법이 어떤 방법을 통해 신뢰성 있고 견고하며, 투명하게 사용되었는지 설명하는 문서가 필요하다. 검토 중인 화학물질의 성질을 적절하게 기술하는 WoE 결합 데이터와 이에 대한 명확한 근거도 함께 제시한다면, 추가 필요한 정보는 더 이상 제공하지 않아도 된다.

4.2. 법령 상 적용 시 유의사항

등록서류는 과학적 논증에 기반한 문서에 바탕을 두고 있는 표준 정보 요건을 채택하기 위해 반드시 문서화 되어야 하고 타당한 근거를 제시할 수 있어야 한다.

5. 활용 예시

QSAR, read-across 및 생체외 시험(*in vitro*) 등 다수의 유사물질 또는 다수의 대체시험자료가 존재할 경우 WoE를 활용하여 대표 시험자료를 선정할 수 있다.

예시 1

[예시 1]과 같이 화학물질 A에 대한 시험자료는 없으나 구조 유사물질 A-1, A-2, A-3에 대한 시험자료가 존재하는 경우, 각 자료에 가중치를 부여하여 신뢰도를 평가한 후 해당 대체시험자료의 결과로 종합적인 평가를 수행하고 화학물질 A에 대한 유해성을 결정하는 사례는 다음과 같다.

화학물질 A의 피부 부식성/자극성 시험자료를 확인하였다. 우선 WoE 절차에 따라 해당 시험항목과 관련 있는 모든 정보를 수집하고, 이를 신뢰성, 시험법, 데이터의 수준 및 시험결과 등을 고려하여 가중치를 합산하고 종합적인 평가를 수행하였다.
i) 정보 수집 화학물질 A의 피부 부식성/자극성 시험은 세 가지 read-across 자료로 구성되어 있으며, 각 시험자료에 대한 세부 정보는 다음과 같다. **[자료 1]** • 시험항목: 피부 부식성/자극성(*in vivo* study) • 시험방법: 래빗(New Zealand White)의 피부에 물질 A-1 0.5 mL의 용량으로 4시간 노출시킨 후 1, 24, 48 및 72시간 동안 관찰 • 시험동물 연령/체중(시작점): 12-16주/2.19~.49 kg • 시험결과: 전체 3마리의 시험동물에서 홍반 점수 0~0.3, 부종 점수 0으로 피부에 자극을 유발하지 않음 • 시험법: OECD Guideline 404(Acute Dermal Irritation/Corrosion) • 노출경로: 피부 도포(반폐색) • 시험 유형: Read-across(카테고리 접근법) • Scoring 시스템: Draize scoring system • 순도 정보: 없음 • GLP 준수 여부: 예 • 신뢰도: 2 • 시험 연도: 1989년

[자료 2]

- 시험항목: 피부 부식성/자극성(*in vivo* study)
- 시험방법: 래빗의 피부(New Zealand White)에 물질 A-2 0.5 g의 용량으로 25시간 노출시킨 후 72시간 동안 관찰
- 시험동물 연령/체중(시작점): 정보 없음
- 시험결과: 전체(수컷/암컷) 6(3/3)마리의 시험동물에서 홍반 점수 0, 부종 점수 0으로 피부에 자극을 유발하지 않음
- 시험법: OECD Guideline 404 유사시험, FHSA(Federal Hazardous Substances Control Act), 16 CFR 1500.41
- 노출경로: 피부 도포(폐색)
- 시험 유형: Read-across(카테고리 접근법)
- Scoring 시스템: Draize scoring system
- 순도 정보: 없음
- GLP 준수 여부: 아니오
- 신뢰도: 2
- 시험 연도: 1991년

[자료 3]

- 시험항목: 피부 부식성/자극성(*in vivo* study)
- 시험방법: 래빗(New Zealand White)의 피부에 물질 A-3 0.5 mL의 용량으로 24시간 노출시킨 후 72시간 동안 관찰
- 시험동물 연령/체중(시작점): 2.5~.5 kg
- 시험결과: 전체 6마리(수컷)의 시험동물에서 홍반 점수 0.22, 부종 점수 0으로 피부에 자극을 유발하지 않음
- 시험법: OECD Guideline 404 유사시험, Journal Officiel de la Republique Francaise of 21/4/71 and 5/6/73
- 노출경로: 피부 도포(폐색)
- 시험 유형: Read-across(카테고리 접근법)
- Scoring 시스템: Primary irritation index
- 순도 정보: 없음
- GLP 준수 여부: 아니오
- 신뢰도: 2
- 시험 연도: 1976년

ii) 종합적 평가

시험물질의 정보, 시험종 특성(*in vivo* study의 경우), 연구 설계에 대한 기술, 문서화된 결과, 연구 설계 및 결과의 타당성 등에 따라 가중치를 합산하여 다음과 같이 신뢰성을 평가할 수 있었다(가중치 합산 시, 평가 항목의 해당여부에 따라 1 또는 0으로 점수를 부여하되, 시험에 따라 부가 가중치가 필요한 경우에는 1/0.5/0으로 차등 부여).

상기 세 가지 read-across 시험자료는 비록 GLP 시험자료는 아니나 신뢰도 평가결과 OECD 가이드라인 404와 유사한 시험방법에 따라 수행된 신뢰도 높은 시험자료로

확인되었다.

세 가지 시험결과에서 노출 초기에 매우 약한 피부 홍반이 관찰되나 이후 피부 자극성이 관찰되지 않았으므로 화학물질 A는 피부 자극성물질로 분류되지 않는 것으로 평가하였다.

[화학물질 A의 평가 기준 항목별 가중치 합산]

평가 기준	평가 항목	자료1	자료2	자료3
시험물질 정보	물질 기본정보(물질명, CAS 번호, 구조식)	1	1	1
	순도	0	0	0
	물질 공급처 정보	0	0	0
	물리화학적 특성	1	1	1
시험종 특성	시험종[a]	1	1	1
	성별[b]	0	1	1
	연령/체중(시작점)[c]	1	0	0.5
연구설계 기술	노출경로	1	1	1
	노출농도	1	1	1
	노출기간 및 관찰시점	1	1	1
	음/양성대조군 여부	1	1	1
	시험동물 수	1	1	1
문서화된 결과	시험법[d]	1	0.5	0.5
	GLP 준수여부	1	0	0
	모든 시험항목에 대한 결과 기술	1	1	1
	적절한 통계처리	0	0	0
연구설계 및 결과의 타당성	시험물질의 특성과 관련하여 결과를 도출할 수 있는 적절한 연구설계인가?	1	1	1
	신뢰할 수 있는 정량적 연구결과인가?	1	1	1
합계		14	12.5	12

[a]시험종: 우선 시험종(1), 일반 시험종(0.5), 적용 불가 시험종 또는 자료 없음(0)으로 차등부여

[b]성별: 피부 부식성/자극성 시험의 경우 시험동물의 성별을 고려하지 않으므로, 정보 여부에 따라 1/0 부여(피부 감작성 및 생식독성 등과 같은 시험은 암컷을 사용하는 것을 원칙으로 하므로, 암컷(1), 암/수컷 평균(0.5), 자료 없음(0)으로 차등 부여)

[c]연령/체중(시작점): 연령/체중(1), 연령 또는 체중(0.5), 자료 없음(0)으로 차등 부여

[d]시험법: 공인된 시험법(1), 유사 시험법(0.5), 공인되지 않은 시험법 또는 자료 없음(0)으로 차등 부여

ⅲ) 결론

화학물질 A의 구조 유사물질 3종 모두 *in vivo* 피부 자극성 시험에서 피부자극이 관찰되지 않았으므로 화학물질 A는 피부 자극성 물질로 분류되지 않는 것으로 판단하였다.

각 항목별로 시험자료의 가중치를 합산한 결과, 자료 1, 자료 2 및 자료 3의 가중치는 각각 14, 12.5 및 12점으로 도출되었다. 이에 따라 화학물질 A의 피부 부식성/자극성 시험에 대해 WoE를 적용하여 가중치를 합산한 결과, 합산 결과가 가장 높은 '자료 1'을 대표자료로 선정하였다.

예시 2

[예시 2]와 같이 화학물질 B를 포함하여 대해 다양한 대체시험자료가 존재하는 경우, 각 자료에 가중치를 부여하여 신뢰도를 평가한 후 해당 대체시험자료의 결과로 종합적인 평가를 수행하고 화학물질 B에 대한 유해성을 결정하는 사례는 다음과 같다.

화학물질 B에 대한 어류 급성독성 시험 자료를 물질 차체의 시험자료, read-across 및 QSAR를 통해 확보하였다. WoE 원칙에 따라 수집된 모든 정보의 신뢰성, 시험법, 데이터의 수준 및 시험결과 등을 고려하여 종합적인 평가를 수행하였다.

i) 정보 수집

화학물질 B의 어류 급성독성 대체시험 자료는 다음과 같이 1개의 시험자료, 두 가지 read-across 및 QSAR 예측 자료로 구성되어 있다.

화학물질 B의 어류 급성독성시험 자료(예시)

시험항목	시험방법	시험결과	신뢰도
어류 급성독성 (자료1)	• 시험법: 없음 • 시험종: *Pimephales promelas* • 노출방법: 지수식 • 노출농도: 0, 10, 100 mg/L • 노출기간: 24시간	LC_0 ≥ 100 mg/L (설정농도)	3 시험자료
어류 급성독성 (자료2)	• 시험법: OECD Guideline 203 • 시험종: *Danio rerio* • 노출방법: 지수식 • 노출농도: 0, 0.1, 1 mg/L • 노출기간: 96시간	LC_{50} > 0.072 mg/L, NOEC ≥0.072 mg/L (측정농도)	2 read-across
어류 급성독성 (자료3)	• 시험법: ASTM 2000, E729-96 유사시험 • 시험종: *Lepomis cyanellus* • 노출방법: 지수식 • 노출농도: 0, 0.01, 0.1, 1, 2 mg/L • 노출기간: 96시간	LC_{50} ≥ 2 mg/L, NOEC ≥ 2 mg/L (설정농도)	2 read-across
어류 급성독성 (자료4)		LC_{50}(96hr) > 0.3 mg/L	3 QSAR

[자료 특성]

수집된 자료는 신뢰도가 2 및 3인 대체시험자료로 구성되어 있으며, 활용된 시험법과 시험종, 방법 및 결과는 다양하며, 각 자료에 대한 정보를 수집한 후 가중치를 합산하였다.

- 시험 자료: 시험방법이 명확하게 제시되어 있지 않고, 공인된 표준 시험법(OECD Guideline 203 등)에서 제시하는 급성 어류독성시험 노출기간(96시간)보다 짧은 24시간 시험자료이므로, 구조 유사물질에 대한 어류급성독성 시험자료를 추가로 확인하였다.

- Read-across 자료: 자료2 및 자료3의 신뢰도는 동일함(신뢰도 2)에 따라 대표자료 선정을 위해 시험법에 따라 가중치를 다르게 부여였다. 즉, 급성 어류독성에 대한 공인된 표준 시험법(OECD Guideline 203)을 활용한 자료2를 ASTM 2000 유사 시험법을 활용한 자료1보다 선순위 선정하였다.

- QSAR 자료: 추가로 EPISUITE 모델을 활용하여 예측결과를 확보하였다. QSAR 결과는 화학물질 B의 어류급성독성 평가결과에 대한 증거력을 보충하는 자료로 활용하였다.

화학물질 B의 평가 기준 항목별 가중치 합산

평가 기준	평가 항목	자료2	자료3	자료1
시험물질 정보	물질 기본정보(물질명, CAS 번호, 구조식)	1	1	1
	순도	0	1	0
	물질 공급처 정보	0	0	0
	물리화학적 특성	1	1	1
시험종 특성	시험종	1	1	1
	성별	0	0	0
	연령/체중(시작점)	1	0	0
연구설계 기술	노출경로	1	1	1
	노출농도	1	1	1
	노출기간 및 관찰시점	1	1	1
	음/양성대조군 여부	1	1	1
	시험동물 수	0	0	0
문서화된 결과	시험법[a]	1	0.5	0
	GLP 준수여부	0	0	0
	모든 독성결과에 대한 결과 기술	1	1	1
	적절한 통계처리	1	1	1
연구설계 및 결과의 타당성	시험물질의 특성과 관련하여 결과를 도출할 수 있는 적절한 연구설계인가?	1	1	1
	신뢰할 수 있는 정량적 연구결과인가?	1	1	0
합계		13	12.5	10

[a]시험법: 공인된 시험법(1), 유사 시험법(0.5), 공인되지 않은 시험법 또는 자료 없음(0)으로 차등 부여

ii) 종합적 평가

화학물질 B의 시험자료, 구조 유사물질 2종, QSAR 결과 모두 어류급성독성이 용해도 한계 이상의 농도까지 LC_{50}이 관찰되지 않았으므로 화학물질 B는 어류급성독성이 낮은 물질로 판단하였다.

각 항목별로 시험자료의 가중치를 합산한 결과, 자료 1, 자료 2 및 자료 3의 가중치는 각각 10, 13 및 12.5점으로 도출되었다. 이에 따라 화학물질 B의 어류급성독성에 대해 WoE를 적용하여 가중치를 합산한 결과, 합산 결과가 가장 높은 '자료 2'을 대표자료로 선정하였다.

iii) 결론

화학물질 B의 어류급성독성 시험결과 비록 표준 노출기간인 96시간보다 짧은 24시간 노출 결과이나 최고 농도에서 시험개체의 사망이 관찰되지 않았다. 2개의 read-across 시험자료는 신뢰도 평가결과 표준 시험방법에 따라 수행된 신뢰도 높은 시험자료로 시험 최고 농도에서 시험개체의 사망이 관찰되지 않았다. 추가로 QSAR 예측결과도 LC_{50}이 용해도 한계 이상으로 확인되었다.

시험자료, read-across 및 QSAR 결과에 근거하여 화학물질 B는 어류급성독성이 낮은 것으로 평가하였다.

6. 참고자료

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- ECHA, Read-across Assessment Framework(RAAF), 2017

- ECHA, Grouping of substances in screening, 2018

- IHCP, A Compendium of Case Studies that helped to shape the REACH Guidance on Chemical Categories and Read Across, 2007

- OECD, WEIGHT OF EVIDENCE ASSESSMENT FOR THE SKIN SENSITISATION POTENTIAL OF 4-ISOPROPYLANILINE (CUMIDINE, CAS 99-88-7), 2014

- EFSA, Guidance on the use of the weight of evidence approach in scientific assessments, 2017

- OECD, Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment, 2019

- OECD, CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT TO INFORM READ-ACROSS OF p-ALKYLPHENOLS: REPEATED-DOSE TOXICITY, 2020

- OECD, CASE STUDY ON THE USE OF INTEGRATED APPROACHES TO TESTING AND ASSESSMENT FOR PREDICTION OF A 90 DAY REPEATED DOSE TOXICITY STUDY (OECD 408) FOR 2-ETHYLBUTYRIC ACID USING A READ-ACROSS APPROACH FROM OTHER BRANCHED CARBOXYLIC ACIDS, 2020

- OECD, Case study on the use of Integrated approaches to testing and assessment for READ-ACROSS BASED FILLING OF DEVELOPMENTAL TOXICITY DATA GAP FOR METHYL HEXANOIC ACID, 2020

[부록] 국외 사례

1. 물질 그룹핑

2. 상관성 방식 활용

Review of Categories presented at OECD Screening Information Assessment Meetings (SIAM) since April 2004 (SIAM 18-22)

Environmental Reviews:

Jane Caley and Daniel Merckel

U.K. Environment Agency

Human Health Reviews:
Meena Sonowane and Amy Benson
U.S. Environmental Protection Agency

Document Coordinators:
Jane Caley, U.K. Environment Agency
Tala Henry, U.S. Environmental Protection Agency

June 2006

Case Study #1 (Limited Health & Full Environment Review)

Category Name: 3 related categories:
ATMP and salts -Phosphonic Acid Compounds Group 1
HEDP and salts - Phosphonic Acid Compounds Group 2
DTPMP and salts - Phosphonic Acid Compounds Group 3

SIAM: 18

Category members:

Sub-category & Chemical name	**CAS No.**
ATMP and salts -Phosphonic Acid Compounds Group 1	
Amino tris(methylenephosphonic acid) (ATMP)	6419-19-8
Amino tris(methylenephosphonic acid), xNa Salt	20592-85-2
Amino tris(methylenephosphonic acid), Na Salt	none found
Amino tris(methylenephosphonic acid), 2Na Salt	4105-01-5
Amino tris(methylenephosphonic acid), 3Na Salt	7611-50-9
Amino tris(methylenephosphonic acid), 4Na Salt	94021-23-5
Amino tris(methylenephosphonic acid), 5Na Salt	2235-43-0
Amino tris(methylenephosphonic acid), 6Na Salt	15505-05-2
HEDP and salts - Phosphonic Acid Compounds Group 2	
1-Hydroxy-1,1-ethane-diphosphonic acid (HEDP)	2809-21-4
1-Hydroxy-1,1-ethane-diphosphonic acid, xNa Salt	29329-71-3
1-Hydroxy-1,1-ethane-diphosphonic acid, Na Salt	17721-68-5
1-Hydroxy-1,1-ethane-diphosphonic acid, 2Na Salt	7414-83-7
1-Hydroxy-1,1-ethane-diphosphonic acid, 3Na Salt	666-14-0
1-Hydroxy-1,1-ethane-diphosphonic acid, 4Na Salt	3794-83-0
1-Hydroxy-1,1-ethane-diphosphonic acid, 5Na Salt	13710-39-9
1-Hydroxy-1,1-ethane-diphosphonic acid, xK Salt	67953-76-8
1-Hydroxy-1,1-ethane-diphosphonic acid, K Salt	17721-72-1
1-Hydroxy-1,1-ethane-diphosphonic acid, 2K Salt	21089-06-5
1-Hydroxy-1,1-ethane-diphosphonic acid, 3K Salt	60376-08-1
1-Hydroxy-1,1-ethane-diphosphonic acid, 4K Salt	14860-53-8
1-Hydroxy-1,1-ethane-diphosphonic acid, 5K Salt	87977-58-0
DTPMP and salts (Phosphonic Acid Compounds Group 3)	
Diethylene triamine penta(methylene phosphonic acid)	15827-60-8
Diethylene triamine penta(methylene phosphonic acid), xNa Salt	22042-96-2
Diethylene triamine penta(methylene phosphonic acid), Na Salt	94987-76-5
Diethylene triamine penta(methylene phosphonic acid), 2Na Salt	94987-75-4
Diethylene triamine penta(methylene phosphonic acid), 3Na Salt	95015-06-8
Diethylene triamine penta(methylene phosphonic acid), 4Na Salt	94987-77-6
Diethylene triamine penta(methylene phosphonic acid), 5Na Salt	61792-09-4
Diethylene triamine penta(methylene phosphonic acid), 6Na Salt	93841-74-8
Diethylene triamine penta(methylene phosphonic acid), 7Na Salt	68155-78-2
Diethylene triamine penta(methylene phosphonic acid), 8Na Salt	95183-54-3
Diethylene triamine penta(methylene phosphonic acid), 9Na Salt	93841-75-9
Diethylene triamine penta(methylene phosphonic acid), 10Na Salt	93841-76-0

Brief description of category:

These are 3 closely-related categories of alkyl phosphonic acids and their sodium or potassium salts. The different salts are prepared by neutralising the acid to a specific pH. The effect of the counter-ion (sodium/potassium) is assumed to be insignificant and the category members will be fully dissociated in dilute aqueous solutions.

The dominant characteristic across all three categories is the presence of several phosphonic acid functions, which can ionise in aqueous solution to phosphonate anions. All category members are highly adsorbing, highly water soluble and have similar use patterns. They all chelate metals ions so they have the potential to disrupt bioavailable concentrations of metallic cations in the blood of fish and invertebrates and to cause nutrient depletion to algae and plants by complexing trace metal cations.

Direct read-across is used for most endpoints. Dermal and irritation studies are considered separately for the acid and salts.

The ATMP category was extended to cover the single sodium salt, although this substance is not commercially produced and has no CAS number.

Category issues raised during comment/SIAM:
The main concern was justification of the conclusion reached for genotoxicity (human health issue). There was also a lot of discussion around the use of data from Industrial Bio-test Laboratories Inc, who were investigated by the US FDA for falsification of results of certain studies, but this is not directly relevant to the category justification.

Generic lessons learned:

- For categories including ionisable compounds, the effect of the counter-ion needs to be considered. It is possible that the counter-ion(s) may pose hazards of greater concern than the common cation or anion on which the category is based (e.g., metal counter-ions that are inherently hazardous on their own). Under such circumstances, it may be of limited utility to group and assess substances by the component which is expected to have the least effect. In other cases, it may be concluded that effects of the counter-ion are insignificant and therefore need not be taken into account in the assessment.

- When comparing acids and their salts, differences arising from pH effects should be considered. For example, skin and eye irritation are likely to be different for an acid compared with its salt.

- It is possible to extend a category to include substances which are not commercially produced but which are covered by the category boundaries.

- For closely related categories, (in this case, structurally related phosphonic acids) it can be helpful to include a link between them to provide further confidence in the conclusions for each individual category.

Lessons learned specific to the category:

- Health: Category members (acids vs. salts) show a difference in skin and eye irritation effects connected with pH.

- Environment: The discussion of nutrient limitation effects was supported by the use of data from DTPMP and other chelating agents, including EDTA. It was helpful to widen the category approach to 3 sub-categories.

Case Study #2 (Health & Environment Review)

Category Name: Ethylene Glycol

SIAM: 18

Category members:

Chemical name	CAS No.
Ethylene glycol	107-21-1
Diethylene glycol	111-46-6
Triethylene glycol	112-27-6
Pentaethylene glycol	4792-15-8

Brief description of category:
A chain length category based on the oxyethylene unit, (CH2 CH2O). Each category member has a structure $HO(CH2\ CH2O)_nH$ where n = 1, 2, 3, 4, or 5. The category boundary appears to be at n = 6-8, when absorption from ingestion decreases and certain physicochemical attributes change significantly and the materials start to become solids.

Polyethylene Glycol 200 (PEG 200) is used to provide supporting data, mainly for the health assessment. This substance is a mixture with n = 2-8 with an average value of 4. It is used to fill quantitative data gaps for repeated dose toxicity and developmental toxicity, and provides supporting data for acute toxicity and carcinogenicity.

Each category member has a different use pattern, some of which are not interchangeable. For example, the major use of Ethylene glycol is the manufacture of polyethylene terephthalate (PET) whereas the major use of Triethylene glycol is natural gas dehydration.

QSARs are used to support the toxicity and ecotoxicity data.

Category issues raised during comment/SIAM:
For human health, QSAR estimations were included. However, several comments suggested that there was not enough discussion of the models in the Dossier; thus, the commenters suggested the estimations be included only in an Appendix.

PEG 200 seems to have a different genotoxicity profile compared with most of the members of the category, although tetraEG did have some equivocal chromosomal aberrations data.The submitter proposed additional genetic toxicity testing (i.e., a CHO/HGPRT assay on pentaEG). However, given the availability of data for the category members, the additional test did not seem necessary. If the testing was still to be done, it should be described as a post-SIDS activity.

The acute Daphnia toxicity of TetraEG does not appear to fit the trend in the ecotoxicity data, showing higher toxicity than expected. However, new data on brine shrimp confirmed low toxicity across the group.

Generic lessons learned:
- Category members need not have similar uses. Under REACH, different industry sectors may need to co-operate on category assessments.

- Surrogate chemicals used to provide supporting data can be a mixture (which can include some/all of the category members). Composition and purity of the surrogate chemical must be clearly stated and its use as a surrogate clearly justified. Further, the surrogate may not be appropriate surrogate for all chemicals or endpoints.

- Additional testing should be described as post-SIDS work if all SIDS endpoints have been met. If all endpoints have not been met, the case should not be presented at a SIAM.

- Because QSAR models for human health endpoints are not as accepted as they are for ecotoxicity endpoints, additional guidance on the use of QSARs for filling data gaps should be developed.

Lessons learned specific to the category:

- Health: Other than tetraEG, which did have some equivocal chromosomal aberrations data, PEG 200 had a different genotoxicity profile than most members of the category and therefore, is not a good surrogate for this endpoint.

- Environment: None

Case Study #3 (Health Review Only)

Category Name: Propylene Glycol Phenyl Ether (isomers)

SIAM: 18

Category (chemical) members:

Propylene Glycol Phenyl Ether	CAS No.
alpha isomer, secondary alcohol	770-35-4
beta isomer, primary alcohol	4169-04-4
mixture	41593-38-8

Brief description of chemical (not a true category):
Monopropylene glycol ethers may exist in two isomeric forms, alpha and beta. The alpha form (a secondary alcohol) is thermodynamically favoured during synthesis and accounts for the majority of the glycol ether mass. The beta form (a primary alcohol) is an impurity in the synthesis process.

All tests were conducted on the commercial mixture, which contains >85% alpha isomer and <15% beta isomer. The individual isomers are not separated nor produced as individual chemicals. A category approach was not used since data were available on the commercial mixture.

Category issues raised during comment/SIAM:
There was some confusion regarding which chemical(s) were being sponsored versus which chemical(s) were tested. This confusion stems from the fact that the commercial product is commonly referred to as CAS# 770-35-4 (for the alpha isomer) and the CAS# assigned to the commercial mixture (41593-38-8) is not commonly used. The commercial product is listed under both CAS#s because modern production methods result in alpha isomer content in excess of 85% and beta isomer content less than 15% and the individual isomers are not separated nor produced as individual chemicals.

Additional clarity on the identity of the commercial substance and the substance tested were suggested, for example:
- specifying typical concentration range of alpha- and beta-isomers
- specifying which compound(s) were being sponsored versus which compound(s) were tested (i.e., the alpha isomer CAS number sponsored, as in the SIAP, but testing was conducted using the commercial mixture).
- establishing, in the documents, that the toxicity, physicochemical and other relevant properties of the various forms are similar and are suitable for consideration as a single set or that the amount of the beta isomer is insignificant compared to the alpha isomer (either should be adequately supported with relevant data).

Generic lessons learned:
- Information for SIDS cases with multiple CAS numbers that do not constitute a category need to be very clearly described so it does not appear that a category approach has been used, when it has not.

Lessons learned specific to the category:
- Health: None; category approach was not used.

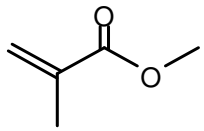
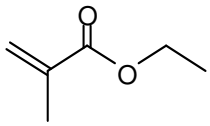
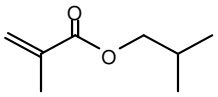
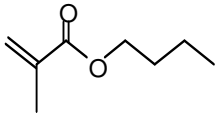
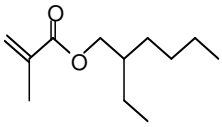
- Environment: Not Reviewed

Case Study #4 (Health and Environment Review)

Category Name: Short Chain Alkyl Methacrylate Esters

SIAM: 18

Category members:

Chemical Name	Synonyms	CAS Number	Structure
Methyl Methacrylate *(Reference chemical for category)*	MMA; Methacrylic Acid, Methyl Ester; Methyl Methacrylate Monomer; Methyl, 2-methyl-2-Propenoate	80-62-6	
Ethyl Methacrylate	EMA; Methacrylic Acid, Ethyl Ester; Ethyl Methacrylate Monomer; Ethyl, 2-methyl-2-Propenoate	97-63-2	
Iso-Butyl Methacrylate	i-BMA; Methacrylic Acid, I-Butyl Ester; Iso-Butyl Methacrylate Monomer; Iso-Butyl, 2-methyl-2-Propenoate	97-86-9	
n-Butyl Methacrylate	n-BMA; Methacrylic Acid, n-Butyl Ester; n-Butyl Methacrylate Monomer; n-Butyl, 2-methyl-2-Propenoate	97-88-1	
2-Ethylhexyl Methacrylate	2-EHMA; Methacrylic Acid, 2-Ethylhexyl Ester; 2-Ethylhexyl Methacrylate Monomer; 2-Ethylhexyl-2-methyl-2-Propenoate	688-84-6	

Brief description of category:
The category is defined as methacrylate esters of straight and branched C2 to C8 alcohols. A chain length category based on the methacrylate ester functional group. This is a standard chain length category, with trends of increasing Log Kow and boiling points with increasing molecular weight and decreasing water solubility and vapour pressures with increasing molecular weight.

MMA was used as a robust reference chemical for the category.
Methyl methacrylate (MMA), the C1 ester, is data rich and already has an agreed SIAR (SIAM #11) and E.U. Risk Assessment.

Based on their clear structure activity relationship with respect to environmental toxicity, distribution and fate, and metabolism and toxicity in mammalian systems, these chemicals are considered together as a category.

- Health:

- The category is also a metabolic pathway category since short-chain alkyl-methacrylate esters are very rapidly metabolized by non-specific carboxylesterases to methacrylic acid and the structurally corresponding alcohol in several tissues.
- The half-life of disappearance of the parent ester from the body is in the order of minutes.
- Data on metabolites were used for the repeated toxicity and developmental endpoints.

- Environment:
  - Toxicity to aquatic organisms increases with increasing lipophilicity and molecular weight.
  - EPIWIN/ECOSAR estimated values were not used to support ecotoxicity data.
  - One category member, 2-EHMA is recommended as a candidate for further work for the environment, due to its potential for bioaccumulation and aquatic toxicity.

Category issues raised during comment/SIAM:
A stronger justification needs to be provided for using data for the reference substance, methyl methacrylate (MMA), for the four category members that lack data. Close structural analogues, structural activity trends, and common metabolism pattern of the parent ester to methacrylic acid and corresponding alcohol have been used to address the endpoints for those chemicals lacking these data. In particular, SIAM wanted a more detailed justification of the use of a category approach and the use of MMA as a predictor for carcinogenicity for the category members.

A concern was raised regarding the use of supporting data on an analogue chemical that was assessed at a previous SIAM. The issue was whether the recommendations for the category members need to be consistent with the supporting analogue chemical. In this case, the analogue data was not heavily relied upon for the category members and the conclusions for the category members were based on data and properties that were different from the analogue chemical. Thus, the conclusion for the analogue (MMA) is not appropriate for these esters.

Generic lessons learned:
- When formulating a category, industry should look for other structurally-related substances which may fit, not just those substances produced by the consortium.

- A category may be justified on more than one basis, for example both a chain length and metabolic pathway category.

- The category chemicals need to be brought together to identify the trends in behaviour. If data indicate there is trend in toxicity, for example, it needs to be stated accordingly.

- Including a chemical that has already been evaluated/assessed can increase confidence in a category. However, strong justification for using analogue data is needed.

- Even when category justification appears robust, it is possible to reach different conclusions for each category member based on biological/toxicological endpoints in different species (e.g. mammalian vs. fish) or based on a trend that is revealed in chemical or toxicological properties. (In this case, this is due to a trend of increasing aquatic toxicity and bioaccumulation potential with increasing molecular weight ended with the conclusion that the highest molecular weight member is a priority for further work, whereas the other members are not.)

- Should consistency should be maintained in conclusion/recommendation for an analogue chemical that has been discussed in earlier the SIAM if the analogue is used to support the majority of category chemicals and when no data are available on any of the category chemicals.

Lessons learned specific to the category:

- Health: A stronger justification needs to be provided for using data for the reference substance, methyl methacrylate (MMA), for the four category members where they lack data.

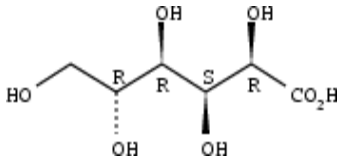
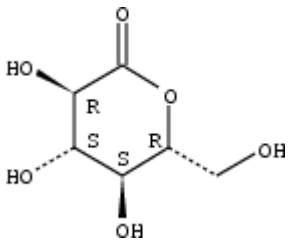
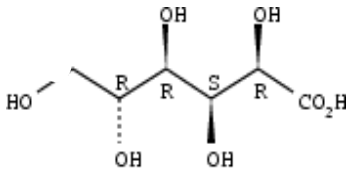
- Environment: One category member, 2-EHMA is recommended as a candidate for further work for the environment, due to its potential for bioaccumulation and aquatic toxicity (i.e., because it represented a "high-end" of a trend of increasing toxicity and bioaccumulation potential).

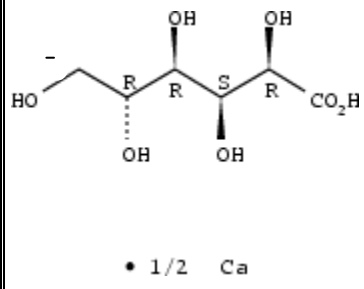
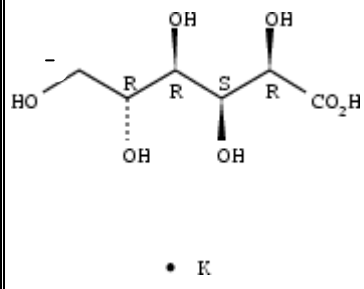
Case Study #5 (Environment Review Only)

Category Name: Gluconates

SIAM: 18

Category members:

Chemical Name	CAS No.	Structural formula
D-Gluconic acid	526-95-4	
Glucono-delta-lactone	90-80-2	
Sodium gluconate	527-07-1	 • IIa

Calcium gluconate	299-28-5 18016-24-5	
Potassium gluconate	299-27-4	

Brief description of category:

The category is an 'acid & salt category 'and focuses on the gluconate anion. Gluconic acid and its mineral salts freely dissociate to the gluconate anion and the respective cations. Glucono-delta-lactone (GDL), the 1,5-inner ester of gluconic acid, is formed from the free acid by the removal of water. On the basis of these spontaneous chemical rearrangements, glucono-delta-lactone, gluconic acid and its sodium, calcium and potassium salts are considered as a category, with all members sharing the same representative moiety, the gluconate anion.

The assessment focuses on the gluconate anion and does not deal with specific effects of the cations. However, for repeated dose toxicity, potential side effects were attributed to high doses of cation intake.

Gluconic acid also has another inner ester, the 1,4-lactone, which was not included in this category. It is not stated why this lactone was excluded but following questions from other member countries, it was stated that the 1,4-lactone is of no commercial interest.

Category issues raised during comment/SIAM:
It would not be possible to extend the group to other substances where the counter ion may be more toxic. Indeed, in some ways it seems less useful to group substances by the component which is expected to have the least effect.

Further justification required for inclusion of the glucono-delta-lactone, taking into consideration metabolic fate and proportion of the metabolic product. [This question highlights that this is also a metabolic category].

Generic lessons learned:
- For categories including ionisable compounds, the effect of the counter-ion needs to be considered. It is possible that the counter-ion(s) may pose hazards of greater concern than the common cation or anion on which the category is based (e.g., metal counter-ions that are inherently hazardous on their own). Under such circumstances, it may be of limited utility to group and assess substances by the component which is expected to have the least effect. In other cases, it may be concluded that effects of the counter-ion are insignificant and therefore need not be taken into account in the assessment.

- A category may be justified on more than one basis, for example both a chain length and metabolic pathway category.

- Substances which are good analogues for a category, should be included to strengthen a category. Such analogues need not be HPV chemicals (i.e. have no commercial interest).

Lessons learned specific to the category:

- Health: For category members with a common cation or anion, the effect(s) of the counter-ion needs to be considered and discussed.

- Environment: For category members with a common cation or anion, the effect(s) of the counter-ion needs to be considered and discussed.

Case Study #6 (Health & Environment Review)

Category Name: Maleic Acid and Malic Anhydride

SIAM: 18

Category members:

Chemical name	CAS No.
Maleic acid	110-16-7
Maleic anhydride	108-31-6

Brief description of category:
Category is based on metabolic series approach. Maleic anhydride is readily hydrolyzed to maleic acid under aqueous conditions and thus these two chemicals are considered analogous. The only difference is that maleic hydride has immediate effects at the site of contact due to the exothermic reaction from the hydrolysis of maleic anhydride to maleic acid and also has a potential to form haptens by acylating with amino acids, resulting in an immunological response.

Maleic acid is an intermediate in the production of maleic anhydride. Most of the maleic anhydride is used in unsaturated polyester resins and smaller amounts are used in production of fumaric and malic acid, as additives, copolymers, and agricultural chemicals, etc.

Mammalian toxicity studies (reproductive, developmental, and in vivo chromosomal aberrations) conducted with maleic anhydride are used to infer toxicity of maleic acid. Ecotoxicological study results reflect pH-dependant toxicity because of maleic anhydride being converted to and measured as maleic acid.

QSARs were not used to support any toxicity and ecotoxicity data.

Category issues raised during comment/SIAM:
Maleic acid has two isomers, cis and trans. However, information is available only on the cis isomer and no information was available on the trans isomer whether it exists as a commercial product or has been isolated as a testing substance.

For the environmental ecotoxicity testing concern regarding the origin of toxicity seen in the maleic anhydride tests was raised; was this due to low pH (reaction of parent with water to form maleic acid as discussed above) only, or was it a combination of pH and the chemical's intrinsic toxicity? Comparison with buffered tests indicated that pH alone was the cause.

Generic lessons learned:
- Structural formulas for category members need to be included.

- For categories including ionisable compounds, either as original category members or degradation products it is essential to differentiate between toxicity stemming from pH effects alone and a combination of pH effects and inherent toxicity. Comparison of unbuffered test results with tests results that include pH buffering (in accordance with OECD test guidelines) is preferred.

Lessons learned specific to the category:
- Health: None

- Environment: Maleic anhydride rapidly hydrolyses to the category member maleic acid, so it is essential to differentiate between toxicity stemming from pH effects alone and a combination of pH

effects and inherent toxicity. In this case this was satisfactorily done by comparison of results from aquatic tests performed with and without pH buffering.

Case Study #7 (Health Review Only)

Category Name: Higher Olefins

SIAM: 19

Category members:

Chemical name	CAS No.
Hexene	25264-93-1
Heptene	25339-56-4
Octene	25377-83-7
Nonene	27215-95-8
Decene	25339-53-1
Dodecene	25378-22-7
Alkenes, C10-13	85535-87-1
1-Hexadecene	629-73-2
1-Octadecene	112-88-9

Brief description of category:
The category consists of nine members with carbon numbers from C6 to C18 mono-olefins (C6, C7, C8, C9, C10, C12, and C10-13 internal olefins and C16 and C18 linear olefins). The internal olefins are predominantly linear with a small amount of branched material as impurities. The data indicate that changing carbon number, location of the double bond or addition of branching does not alter the mammalian health and biodegradation endpoints and helps to indicate increasing or decreasing trends for ecotoxicity. The data indicate an increasing or decreasing trend from the shortest alpha or internal olefin (C6) to the longest alpha or internal olefin for various physicochemical properties and ecotoxicity endpoints, whereas there is no apparent critical differences across category members for biodegradation and health effect endpoints. Therefore, data for linear alpha olefins and linear and branched internal olefins are used to characterize the human and environmental health hazards for this category.

QSARs were used to support ecotoxicity data.

Category issues raised during comment/SIAM:
Many more higher olefins exist in the OECD HPV Database and since data for some non-HPV substances were used as supporting data for this category, the question was raised why not bring forward such other non-HPV chemicals (29 are listed in the comment) under this category. Although this concept appears well-founded, the chemicals in this category are sponsored by at least one member company of the consortium. Because of the substantial amount of work that is involved in preparing the necessary documents, if the producers of the other chemicals are willing to sponsor their chemicals, the Higher Olefins consortium is willing to work with other higher olefin producers.

Identity of category members needs to be clearly defined as to the proportion of branched components and degree of branching.

Generic lessons learned:
- All category members need to be clearly identified, including proportion of branched components and degree of branching.

- Inclusion of other structurally related chemicals with similar properties that are not included in the proposed category will be beneficial in assessing hazard potentials of a large number of chemicals as

a group. The consortium needs to check with other producers/manufacturers for providing appropriate support and information.

Lessons learned specific to the category:

- Health: None

- Environment: Not Reviewed

Case Study #8 (Health & Environment Review)

Category Name: Monoethylene Glycol Ethers

SIAM: 19

Category members:

Chemical name	CAS No.
Ethylene glycol propyl ether (EGPE)	2807-30-9
Ethylene glycol butyl ether (EGBE)	111-76-2
Ethylene glycol butyl ether acetate (EGBEA)	112-07-2
Ethylene glycol n-hexyl ether (EGHE)	112-25-4

Brief description of category:
All category members have similar molecular structures with a backbone of glycol ether. The only difference in these molecules is in the alkyl functional groups —propyl, butyl, or hexyl, and whether or not the hydroxyl group is free or acetylated. Ethylene glycol butyl ether acetate—the acetylated glycol ether—metabolizes rapidly in vivo to the corresponding ethylene glycol butyl ether, making it adequate for the inclusion in the category and thus it is reasonable to extrapolate mammalian toxicity data for the corresponding ether to address the data gaps for the acetate. However, in the environment, conversion of ethylene glycol butyl ether acetate is not expected to be rapid. For this reason, ecotoxicity data for the corresponding ether cannot be extrapolated to the acetate. Thus in addition to the glycol ether data, all ecotoxicity data for the acetate have been provided.

Use patterns for all category members appear to be the same, with associated emissions predicted to be similar.

EPIWIN/ECOSAR estimated values have been used to support ecotoxicity data.

Category issues raised during comment/SIAM:
EGBE data are used throughout the assessments to support the category member EGBEA, for which there were data gaps. However, it is not formally a part of the category as it already has a SIDS dossier and SIAR that were agreed upon at SIAM 6 (sponsored by Australia). Data used for the substance needs only to be drawn from this source.

Suggestions were made to update EGBE dossier with available new information e.g., new cancer data and new ecotoxicity information; however, the purpose of this dossier is not meant to be an update for EGBE. In addition, carcinogenicity is not a SIDS endpoint and as mentioned in the category justification, EGBE ecotoxicity data are not needed for EGBEA because aquatic toxicity of EGBE is different from that of EGBEA and could not be extrapolated to EGBEA. All endpoints for EGBEA have addressed by experimental data. It was suggested that an inclusion of the recently conducted repeated-dose (inhalation) toxicity study on EGBE should not be reviewed since the EGBE dossier was agreed at SIAM 6. However, this study was conducted by more relevant route of exposure and therefore, was appropriate to include in this dossier.

Metabolism of EGBEA was partially explained in the SIAR based on EGMEA and EGEEA; a suggestion was made to include those robust summaries in the IUCLID. However, general information about other glycol ethers that are not the category members would not have made the category robust.

Clarification was suggested in the statement of how acute toxicity changes with molecular weights (e.g., oral LD50 values in rats for all category members range from 739 (EGHE) to 3089 mg/kg bw (EGPE), with values increasing with decreasing molecular weight).

When using data for a chemical previously assessed at the SIAM, it needs to be verified whether the same studies drew similar conclusions. For example, NOAELs derived for reproduction toxicity should be similar in both dossiers.

When documenting uses, include specific uses of individual category chemicals, if available, and varied.

The purity/impurity information of the marketed substance needs to be indicated in Dossier sections 1.1-1.4. This section encompasses all synonyms. When the test substance is indicated as "other", then it needs to be specified in the appropriate robust summary and its purity/impurity information needs to be included.

Generic lessons learned:

- When making statements regarding how toxicity varies among the category members, be more specific, e.g., provide information if there is a trend in toxicity and molecular weight, etc.

- Purity/impurity information of the tested substance needs to included in the SIDS dossier, when the "other" tested substance is used in the study and the specific information is not covered under sections 1.1-1.4.

- When using a chemical that has been assessed previously (i.e. agreed in a previous SIAM) as a supporting chemical, the documents need to be clear about which chemical(s) are 'category members' and which chemical(s) are 'supporting chemical(s).

- When using a chemical that has been assessed previously (i.e. agreed in a previous SIAM) as a supporting chemical, conclusions drawn based on the supporting chemical must be consistent with conclusions drawn in the earlier assessment/SIAM. OECD needs to develop cross checking/referencing tool (This could also apply under REACH when using supporting data from a chemical which has already been registered and evaluated).

- New information generated on a chemical that has been assessed in an earlier SIAM should be included in the dossier being presented, if that information is relevant to address a particular SIDS endpoint for any/all chemical(s) in the current dossier. Previously submitted information generated on a chemical that has been assessed in an earlier SIAM need not be included as a category member and data used for the substance needs only to be drawn from the previous dossier.

Lessons learned specific to the category:

- When structurally related ethers and ether acetates are proposed as a single category, data (trends) should be assessed to determine if splitting into ether and acetate sub-categories is more appropriate for relevant endpoints/sections.

- Health: None

- Environment: An example of the "sub-group" approach within a category for specific endpoints. As described, all members are ethylene glycols; three category members are glycol ethers but one is a glycol ether acetate. The acetate was considered separately from the ethers, as the acetate group is only slowly hydrolysed at environmental pH to the alcohol. This approach seems warranted as the departure from the common alcohol functionality displayed by the majority of category members is fairly great (alcohol to acetate), and in addition the acetate appeared to be more acutely toxic.

Case Study #9 (Health & Environment Review)

Category Name: Linear Alkylbenzene Sulfonates

SIAM:20

Category members:

Chemical name	CAS No.
Decylbenzene sulfonic acid, sodium salt	1322-98-1
Dodecylbenzene sulfonic acid, sodium salt	25155-30-0
Tridecylbenzene sulfonic acid, sodium salt	26248-24-8
Undecylbenzene sulfonic acid, sodium salt	27636-75-5
C_{10-16} Monoalkylbenzene sulfonic acid, sodium salt	68081-81-2
C_{10-13} Alkylbenzene sulfonic acid, sodium salt	68411-30-3
C_{10-14} Alkyl deriv benzene sulfonic acid, sodium salt	69669-44-9
C_{10-14} Monoalkylbenzene sulfonic acid, sodium salt	85117-50-6
C_{10-13} Alkyl deriv benzene sulfonic acid, sodium salt	90194-45-9
4-C_{10-13}-sec Alkyl deriv benzene sulfonic acid, sodium salt	127184-52-5

Brief description of category:
The LAS molecule contains an aromatic ring sulfonated at the para position and attached to a linear alkyl chain at any position other than at the terminal carbons. The alkyl chain usually has 10 to 14 carbon atoms; and they are 87 to 98% linear. Although commercial LAS consists of more than 20 unique components, the ratio of the various homologues and isomers (which represent different alkyl chain lengths and aromatic ring positions on the linear alkyl chain) is fairly constant in current products; the weighted average carbon number of the alkyl chain based on production volume per region is between 11.7-11.8.

LAS is a category because of the similarity of the different mixtures, the commercial uses, the fate endpoints, the health effects, and environmental effects. It is the primary cleaning agent used in many laundry detergents and cleaners, and is present at concentrations up to 25 percent in consumer products, and up to 30 percent in commercial products, with the exception of one reported product, which is a 45 percent concentrated solid form that is mechanically dispensed into diluted solution for dishwashing.

As expected with a chain length category, trends in properties are seen with increasing alkyl chain length. For example, Kd values for LAS increase with increasing alkyl chain length of LAS homologues and measured fish BCFs generally increase with increasing alkyl chain length. Aquatic toxicity is greater for LAS with longer carbon chains. This is supported by QSAR predictions of aquatic toxicity. The QSARS for acute toxicity were used to normalize the experimental NOEC values to NOECs for C11.6 LAS, the average commercial mixture. [Note – a similar approach was used for the ICCA assessment of Long Chain Alcohols].

Category issues raised during comment/SIAM:
Two members of the category are not HPVs. It was suggested that they not be included, however, the chemicals were retained because convention in the SIDS program has been that non-HPV chemicals can be used in categories to fill certain endpoints.

For substances that are mixtures with different alkyl chain lengths, physical property information (and the trends in physicochemical properties) for the different chain lengths is desirable. This issue applies to categories as well as to mixtures.

Additional composition information was needed (e.g., distribution of alkyl chain lengths) to determine how the members relate to each other within the category.

There was a request for more matrices to determine read-across. However, because the mixtures are very similar to each other, the data matrices were considered unnecessary.

The category members are mixtures, whereas some data are available for more pure compounds. The difference between the structures of the test substances and the category members needs to be discussed.

Aquatic toxicity data is available for both commercial products (mixtures) and pure C13 and C14 homologues. The pure homologues showed higher toxicity than the commercial mixtures but data for the pure homologues was not used to drive the recommendation since they are not commercially supplied.

Member countries requested further justification of the approach for normalising experimental NOECs from aquatic toxicity tests to NOECs for the C11.6 average LAS commercial mixture.

Generic lessons learned:

- Category members need not be HPV chemicals. Good analogue chemicals strengthen the category (i.e. read-across).

- As much detail on product composition (as well as physicochemical properties) as possible needs to be provided so that an independent assessment of the category can be made.

- When category members include both mixtures and pure homologues, the differences between the structures of the test substances and the category members need to be clearly presented.

- For substances that are mixtures with different alkyl chain lengths, physical property information (e.g., distribution of alkyl chain lengths) is needed to determine how the members relate to each other within the category (e.g. identify or discount trends). This issue applies to categories as well as to mixtures.

- Additional guidance is required on how data from pure homologues should or should not be applied to mixtures, particularly in cases where the pure homologue, which is not commercially supplied, shows higher or lower toxicity than the commercial mixture.

Lessons learned specific to the category:

- Health: None

- Environment: None

Case Study #10 (Health & Environment Review)

Category Name: Persulfates

SIAM: 20

Category members:

Chemical name	CAS No.
Ammonium persulfate	7727-54-0
Potassium persulfate	7727-21-1
Sodium persulfate	7775-27-1

Brief description of category:
The category chemicals have similar chemical structure and physicochemical properties. These inorganic substances differ only by the cationic portion of the salt. The anionic part is identical and based on the available data; the potassium and sodium salts are expected to display similar environmental, ecotoxicological and mammalian toxicological properties. Dissolution of the ammonium cation may complicate the toxicological profile of ammonium persulfate.

QSARs were not used to support any toxicity and ecotoxicity data.

Category issues raised during comment/SIAM:
A minor comment was made concerning including acute toxicity values for each category member rather than providing them as a range.

Ammonium persulfate is the "odd one out". Unlike the other two members which when dissociated in solution exist as simple alkali metal cations and the persulfate anion, the ammonium cation will also be in equilibrium with ammonia depending on the solution's pH. Dissolution of ammonium persulfate will also affect pH itself (cf case study #6). This may complicate the toxicological profile of ammonium persulfate.

Generic lessons learned:
- Include individual toxicity values for category members where feasible and possible.

Lessons learned specific to the category:
- Health: None

- Environment: Ammonium persulfate is the "odd one out". Unlike the other two members which when dissociated in solution exist as simple alkali metal cations and the persulfate anion, the ammonium cation will also be in equilibrium with ammonia depending on the solution's pH. Dissolution of ammonium persulfate will also affect pH itself (cf case study #6). This may complicate the toxicological profile of ammonium persulfate.

Case Study #11 (Health & Environment Review)

Category Name: C9 Aromatic Hydrocarbon Solvents

SIAM: 21

Category members:

Chemical name	CAS No.
1,2,4-trimethylbenzene	95-63-6
1,3,5-trimethylbenzene	108-67-8
ethylmethylbenzene (mixed isomers)	25550-14-5
solvent naphtha, (petroleum), light aromatic	64742-95-6

Brief description of category:
The C9 Aromatic Hydrocarbons Solvents Category is first composed of petroleum naphtha hydrocarbon mixture, "Solvent naphtha, (petroleum), light aromatic" (CAS number 64742-95-6), which distils within a narrow boiling range (~160-175ºC). Light Aromatic Solvent Naphtha, hereafter referred to as C9 Aromatic Naphtha, is a mixture of individual isomers that predominantly have 9 carbon atoms with a benzene ring and a limited number of short-chain alkyl constituents. Typical constituents may include: three methyl groups (e.g., 1, 3,5-trimethylbenzene and 1,2,4-trimethylbenzene), an ethyl and a methyl group, one propyl group (e.g., isopropyl benzene), and small percentages of C8 and C10 aromatic hydrocarbons (e.g., mixed xylenes).

This category also includes three of the individual C9 aromatic isomers (1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene, and mixed ethylmethylbenzenes), that have relatively limited production and are used primarily as chemical intermediates. The justification for including the isolated C9 aromatic isomers in the category with the C9 aromatic naphtha is:

(1) Trimethylbenzene (TMB) and ethyltoluene (ET) isomers are major constituents in the C_9 aromatic naphtha. The C_9 aromatic naphtha (CAS# 64742-95-6), which was the subject of a 1985 U.S. TSCA test rule, was required to have a minimum total ET-TMB content of 75%. Commercial C_9 aromatic naphtha typically contains 1,2,4-trimethylbenzene at 20-45%, 1,3,5-trimethylbenzene at 8-15%, and mixed ethylmethylbenzenes at 25-35%.
(2) All category members have similar physicochemical and fate properties.
(3) Existing data indicate that the toxicity of the isolated C_9 aromatic isomer substances is similar to that of the C_9 aromatic naphtha.

Category issues raised during comment/SIAM:
Significant issues were related to category member inclusion and data completeness/adequacy.

Category Member Inclusion Issues:
1) It was suggested that the SIAR, based on "C_9 aromatic hydrocarbon solvents" was too broad and that the conclusions regarding human health should more closely match the available data rather than being attributed to the entire category without a stronger basis.

2) It was suggested that the following three chemicals (that are on the OECD HPV list) to be added to the category (note: the sponsor did not add the chemicals):

611-14-3: 1,2-Ethylmethylbenzene
622-96-8: 1,4-Ethylmethylbenzene
25551-13-7: Trimethylbenzene (mixed isomers)

3) See Case Studies #4 and #5 for related category member inclusion issues.

Data Issues:

1) The physicochemical and fate properties were not available and were not similar for all category members as had been stated in the SIAR. Therefore, the category discussion needs to be clearer as to which data are available and the SIDS documents need to describe cases (e.g., biodegradation) where data were not similar.

2) It was suggested that a ready biodegradability test according to the OECD TG 301 and GLP for the substance 1,2,4-trimethylbenzene (TMB) OR 1,3,5-TMB be conducted to confirm the conclusion on the ready biodegradability of all members of the category. If the new study showed no ready biodegradability, the justification of the category would need to be revised and other biodegradability tests may need to be performed. **As a result, the assessment was not agreed upon and will be presented at a future SIAM for agreement.**

3) Data gaps for genotoxicity were noted. The C9 aromatic naphtha mixture could be about 33% ethylmethylbenzenes and there should be some genotoxicity data on this class of compounds. If there are data, they should be included since the SIAR is not specifically to C9 aromatic naphtha but more broadly, for the C9 aromatic hydrocarbon solvents category. The sponsored mixture also contains significant % of n-propylbenzene and cumene; key genotoxicity endpoints for these chemicals (or at least some discussion ofthem) should be included in the SIAR.

Another commenter noted that the SIDS dossier only contains information on the mammalian toxicity and genotoxicity studies on 1,2,4-TMB and 1,3,5-TMB that are cited in the SIAR (and no acute mammalian toxicity data were included). Toxicity studies on these compounds were only generally used in the SIAR if a particular toxicity end-point was not covered by a study on the mixture (C_9 aromatic naphtha). The SIDS dossier and the SIAR should contain a wider range of data for these compounds because they are category members (and it appears that some acute and genotoxicity data are available for both compounds). The US response was to suggest adding the genotoxicity data if they are adequate.

4) Additional ecotoxicity data should be gathered to support the statement that the toxicity of the isolated C9 aromatic isomer substances is similar to that of the C9 aromatic naphtha (i.e., fish toxicity data were available only for 1,2,4-TMB and C9 aromatic naphtha [CAS No. 64742-95-6] and daphnia and algae toxicity data were available only for C9 aromatic naphtha). Because methylethylbenzenes are a generalised structure of 3 isomers, it was also suggested that two of these isomers (i.e. 1,3-ethyltoluene and 1,4-ethyltoluene; the two isomers present at highest concentration in solvent naphtha) be tested in order to check that the position of the substitution on the benzene ring has no effect on the ecotoxicological profile of the substance. Additional data were to be added for 1,2,4-trimethylbenzene and 1,3,5-trimethylbenzene; these data are expected to cover the ethylmethylbenzenes (as suggested by computer modelling).

5) For mixed ethymethylbenzene, the IUCLID contained data mainly on the substance 1-ethyl-3-methylbenzene (CAS No. 620-14-4). A commenter noted that the other two components should have been taken into account as well. The US Response was to add more data.

6) For mixed ethymethylbenzene, a commenter notes that no mammalian toxicity data were contained in IUCLID – however, the commenter found data to add. The U.S. response was that we would consider adding some information on the additional chemicals.

Generic lessons learned:

- It is important to provide a well-reasoned category justification/discussion.

- It is important to provide all of the data available for each chemical in a category. Generally, broad categories with data primarily on mixtures will require more information and discussion to justify the category.

- It is not appropriate to use data for mixtures in a category to represent single substances in a category (e.g., in the case of biodegradation).

Lessons learned specific to the category:

- Health:

- Environment:

Case Study # 12 (Environment Review Only)
Category Name: Hydrotropes

SIAM: 21

Category members:

Chemical name	CAS No.
Xylenesulfonic acid, sodium salt	1300-72-7
Toluenesulfonic acid, sodium salt	1300-72-7
Xylenesulfonic acid, ammonium salt	26447-10-9,
Cumenesulfonic acid, sodium salt	28348-53-0 and 32073-22-6
Cumenesulfonic acid, ammonium salt	37475-88-0

Supporting Substances:

Chemical name	CAS No.
Xylenesulfonic acid, sodium salt	827-21-4
Xylenesulfonic acid, calcium salt	28088-63-3
Xylenesulfonic acid, potassium salt	30346-73-7
Toluenesulfonic acid, potassium salt	16106-44-8

Brief description of category:
6 category members, 4 supporting substances. Category formed based on structural similarities, use pattern, environmental fate, and (eco)toxicological properties. Category is comprised of 3 sub-groups (methyl, ethyl and 2-propyl substituted benzenes) and a range of positional isomers (varying locant for Me, Et, iPr group on the ring; ortho, meta or para). However the sulfonic acid has a greater effect on physical-chemical properties than stepwise increase in substituent carbon chain length, so read-across from one sub-group to another is deemed valid.

Category issues raised during comment/SIAM:

Generic lessons learned:
- It might be preferable to include all substances that are within the scope of the category (i.e. the supporting substances). Industry's decision not to do so was because the excluded substances were not HPV.

Lessons learned specific to the category:
- Health: Not Reviewed

- Environment: None

Case Study #13 (Health Review Only)

Category Name: Diethylene Glycol Ethers

SIAM: 21

Category members:

Chemical name	CAS No.
Diethylene glycol ethyl ether (DGEE)	111-90-0
Diethylene glycol ethyl ether acetate (DGEEA)	112-15-2
Diethylene glycol propyl ether (DGPE)	6881-94-3
Diethylene glycol butyl ether acetate (DGBEA)	124-17-4
Diethylene glycol n-hexyl ether (DGHE)	112-59-4

Brief description of category:
Using a category approach for screening level hazard assessment for the glycol ethers is appropriate based on similarities in molecular structure and functionality (with a generic molecular structure of $HO(CH_2\ CH_2O)_2\ R$ or $CH_3C(=O)O(CH_2\ CH_2O)_2\ R$), where R = a straight chain alkyl group (ethyl, propyl, butyl, or hexyl). The diethylene glycol ethers contain a free hydroxyl group at the end of the molecule. The diethylene glycol ether acetates differ from the ethers in that the hydroxyl group is esterified with an acetic acid group.

The category members and supporting chemicals demonstrate similar physical and environmental fate properties. The diethylene glycol ethers in the category exhibit a trend in aquatic toxicity (which is also supported using analogues). As the molecular weight and log K_{ow} increase, the toxicity also increases. The same trend can be observed for the acetates. Each group (ethers and acetates) must be treated as subcategories because of the marked differences in functionality and the fact that the acetates only slowly hydrolyse to the alcohol (ether) under environmentally relevant aqueous conditions.

In mammalian systems, the diethylene glycol ethers have similar metabolic pathways – they are poor substrates for alcohol dehydrogenase (Dow Chemical Company, 1982) and good substrates for cytochrome P-450 (Kawamoto *et al.,* 1990). Further, a major metabolite of DGEE, (68% in urine) was (2-ethoxyethoxy)-acetic acid (Miller, 1987); a major metabolite of DGBEA was identified as 2-(2-butoxyethoxy)-acetic acid (Deisinger and Guest, 1985a). Also, DGEEA and DGBEA are included because evidence demonstrates that DGBEA is rapidly hydrolyzed to DGBE by esterases present in blood.

The mammalian toxicities show a pattern consistent with the molecular and metabolic similarities of the chemicals. Valid repeated dose oral toxicity studies conducted with DGEE, DGPE, DGBEA, DGHE and the supporting chemical DGBE (ranging in duration from 30 days to 2 years) generally report kidney and liver toxicity, absolute and/or relative changes in organ weights, and/or some changes in hematological parameters at high doses. The majority of studies show no reproductive or developmental toxicity for the diethylene glycol ethers in this category.

Category issues raised during comment/SIAM:
The category approach was clear and well-documented, with good choices for the supporting chemicals. The similarities show that the chemicals could be appropriately grouped together (although additional discussion of trends was requests; and added). The conclusions for the category outlined in the SIAR and SIAP appear to be sound.

Only selected members of 'diethylene glycol ethers' were included. Absence of diethylene glycol monomethyl ether was noted. Diethylene glycol butyl ether was used only a supporting chemical because it was presented at an earlier SIAM, whereas DGBEA (the acetate) was included in the current category.

Addition of a table listing the reliable studies available for the category members for all health end points would be useful.

Generic lessons learned:

- Future submissions should be as complete as possible and should aim to include the most relevant chemicals for a cohesive category. Incentives for industry sponsors to include all chemicals that are covered by the definition of a category would help achieve this goal.

- Data matrices included in submissions would improve clarity and facilitate easier review.

Lessons learned specific to the category:

- Overall, this category submission [Category - Diethylene Glycol Ethers] was considered to be very good; the category justification and supporting chemicals were well supported, data was complete, and conclusions in the SIAR and SIAP were deemed sound.

- When structurally related ethers and ether acetates are proposed as a single category, data (trends) should be assessed to determine if splitting into ether and acetate sub-categories is more appropriate for relevant endpoints/sections.

- Health:

- Environment:

Case Study #14 (Health & Environment Review)

Category Name: Amine Oxides

SIAM: 22

Category members:

Chemical name	CAS No.
1-Dodecanamine, N,N-dimethyl-, N-oxide	1643-20-5
1-Tetradecanamine, N,N-dimethyl-, N-oxide	3332-27-2
Amines, C10-16-alkyldimethyl, N-oxides	70592-80-2
Amines, C12-18-alkyldimethyl, N-oxides	68955-55-5

Supporting members (later added to the category):

Chemical name	CAS No.
Decanamine, N,N-dimethyl-, N-oxide	2605-79-0
Hexadecanamine, N,N-dimethyl-, N-oxide	7128-91-8
Octadecanamine, N,N-dimethyl-, N-oxide	2571-88-2
Amine oxides, cocoalkyldimethyl	61788-90-7
Amines, C10-18-alkyldimethyl, N-oxides	85408-48-6
Amines, C12-16-alkyldimethyl, N-oxides	85408-49-7
Ethanol, 2,2'-iminobis-, N-coco alkyl derivs., N-oxides	61791-47-7
Ethanol, 2,2'-(dodecyloxidoimino)bis-	2530-44-1
Ethanol, 2,2'-(octadecyloxidoimino)bis-	14048-77-2
Ethanol, 2,2'-iminobis-, N-tallow alkyl derivs., N-oxides	61791-46-6
Ethanol, 2,2'-[(9Z)-9-octadecenyloxidoimino]bis-	93962-62-0

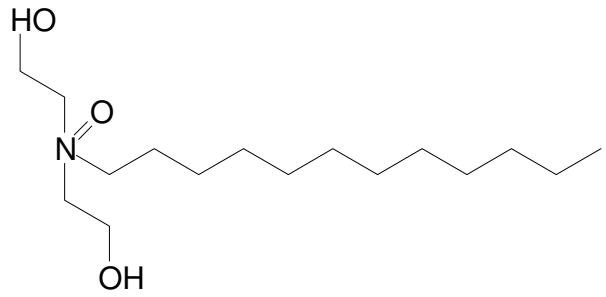
Brief description of category:

The justification for grouping the amine oxides into a category is based on their structural and functional similarity. All of the substances in this category are surfactants, and have a polar "head" (the amine oxide) and a relatively inert hydrophobic "tail" (the long alkyl substituent). The structural variations in the category are three-fold: 1) the nature of the second and third substituents on the amine are either methyl groups or hydroxyethyl groups; 2) the long alkyl chain ranges in length from 8 to 20 carbons; and 3) the long alkyl chain may contain one or two double bonds as in C18:1 (oleyl) or C18:2 (linoleyl).

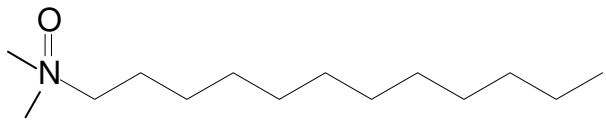
Commercial amine oxides are either alkyl dimethyl or alkyl dihydroxyethyl amine oxides. These two categories contain 2 methyl groups or 2 hydroxyethyl groups, respectively, which are attached to the tertiary nitrogen. Alkyl chain lengths range from 8 to 20. The predominant lengths for these chains are 12 and 14, with average chain lengths for the mixtures of 12.9 to 13.5; one exception regarding the chain length is for the tallow-derived compound. All CAS numbers represent complex mixtures of various chain lengths, with the predominant chain length cited as the chemical name for the category members. The nomenclature and CAS identifications for these amine oxides are based on historical and geographical considerations, not on significant differences in the structure and composition of the commercial substances.

Typical structures for amine oxides are as follows:

C_{12} dihydroxyethyl amine oxide



C_{12} dimethyl amine oxide



The chemical behaviors of the amine oxides are expected to be very similar. Differences relate to the alkyl substituents (i.e., their nature [methyl, hydroxyethyl] and the number of carbon atoms in the alkyl chain [8 to 20]). The presence of methyl- vs. hydroxyethyl-substituents affects the basicity of the nitrogen only marginally, and the hydroxyethyl group lends more bulk to the hydrophilic head-group of the surfactant. The length of the longest alkyl substituent does not alter the chemical reactivity of the molecule, but does affect its physical properties.

Category issues raised during comment/SIAM:
At SIAM it was decided to include the 11 supporting substances in the category, increasing the CAS number count from 4 to 15. There had been some confusion amongst member countries previously about which chemical members constituted the category.

Several potential differences among category members were noted. Characteristics of ethyl and hydroxyethyl members may vary more than just in bulk of head group; hydrophilicity was one possible difference noted. It was also suggested that unsaturated analogues among the hydroxethyl members may react differently. Lack of data to demonstrate that hydroxyethyl compounds have similar behaviour across a range of endpoints was noted.

Due to greater dissimilarity between ethanolamines and the four main amine oxide substances as compared to the other six supporting amine oxides (which are dimethyl amine oxides), it was suggested to split the category into two subcategories (if the supporting substances are to be category members), or minimize read across from the ethanolamines to the main substances (if they are intended to be supporting only). Ecotoxicity and biodegradation data for ethanolamines are quite similar to the dimethyl amine oxides.

For substances that are a complex mixture of various chain lengths, values for certain physicochemical properties should be presented for each carbon chain length present in the complex mixture (substance). Although this has been partially addressed using EPIWIN, some endpoints have been filled by computer modelling but others have not. The values were presented only for those endpoints for which it is considered acceptable to use such modelled data.

The commenter at SIAM suggested that consideration should been given as to how biodegradation rate would vary between components with longer and shorter chain alkyl groups. The US responded that longer chain-lengths are more sorptive and that ready biodegradation seems to apply up to a C17.2 AO, suggesting that biodegradation in soil would apply for the longer chain-lengths (but no data exist to support conclusions regarding variability in biodegradation rates).

In the discussion of chronic toxicity to fish, daphnia, algae, a "normalized" (mean) value for carbon chain length (C12.9) was used. It was suggested that values for each of the category members in the dossier and in the SIAR be added, or at least a range provided, to better represent the actual toxicities observed for individual category members.

It appeared that all category members (i.e. CAS numbers) displayed acute ecotoxicity according to GHS Acute I classification to at least one aquatic organism. This is due to the complex nature of each member, having a range of carbon chain lengths, but all containing a proportion of the more toxic chain length components. For this reason a sub-group approach was not used for the environment, and all members were considered together.

Generic lessons learned:

- Although there is guidance that suggests that values be provided for each category member when presenting the documents at SIAM, the guidance has not been practiced in most cases.

- Information on individual compounds with different carbon chain lengths can be useful supplemental information (measured/modelled) when category members are mixtures.

- Data gaps may be appropriately filled using modelled data in some cases. However, because certain endpoints can be modelled for certain chemicals, does not imply that all data gaps can be filled using a single model (i.e., models are not "one-size-fits-all", rather, they need be applied within the context for which they were designed).

Lessons learned specific to the category:

- Health:

- Environment:
  - Each category member is a complex mixture of chain lengths. As certain chain lengths are more toxic than others and these chain lengths appeared to be present in each category member, all members were considered together for the environmental assessment.
  - Each category member is a complex mixture of chain lengths. A conclusion for biodegradation based on tests on mixtures is not strictly possible to derive according to current OECD guidance. However it may be possible to infer from the results that most of the chain lengths are likely to be readily biodegradable. **This issue is likely to come up time and time again for commercial products under REACH**.

Case Study #15 (Health & Environment Review)

Category Name: Epoxidized Oils and Derivatives (EOD)

SIAM: 22

Category members:

Chemical name	CAS No.
Fatty acids, tall-oil, epoxidized, 2-ethylhexyl esters (ETP)	61789-01-3
9-Octadecanoic acid (Z)-,epoxidized, ester w/propylene glycol (EODA)	68609-92-7
Epoxidized soybean oil (ESBO)	8013-07-8
Epoxidized linseed oil (ELSO or ELO	8016-11-3

Brief description of category:
The category chemicals are epoxidized esters of naturally occurring; long chain fatty acids, primarily the C_{18} acids (oleic, linoleic, and linolenic acids) with primary alcohols, diols, or triols. There is a considerable overlap in the composition of the fatty acid portions of these products. These materials are considered a category for purposes of environmental and health hazard screening assessments because of their similarities in metabolism in microbial, aquatic, and mammalian systems. It is well understood that fats in this category will be metabolized by carboxylesterases of fish, aquatic invertebrates and mammals resulting in a mixture of epoxidized fatty acids and 2-ethylhexanol from ETP, epoxidized fatty acids and propylene glycol from EODA, and epoxidized fatty acids and glycerol from ESBO and ELSO. Being the primary constituents of the metabolic process, the epoxidized fatty oils are considered as representative chemicals to assess potential hazards. Alcohols, the minor constituents of the metabolic products are not produced in sufficient quantities to influence the toxicity profile of the EOD materials. In addition, the hazard profiles of these alcohols have been assessed in previous SIAMS.

QSARs were not used to support any toxicity and ecotoxicity data.

Basically, all EODs are used in plasticizers to keep plastics and rubber soft and pliable. ESBO and ELSO are approved by US EPA for use as inert ingredients in pesticides.

Read-across is important in the category:

Read Across from	**Read Across to**	**Endpoint**
ETP, ESBO, EODA	EODA	Tox. data
ETP	EODA, ESBO, ELSO	Phys-chem
ETP, ESBO	EODA, ELSO	Biodeg., genetic tox., repro tox, developmental tox
ESBO	ETP, EODA, ELSO	acute fish, daphnia and algae
EODA	ETP, ESBO, ELSO	Chronic Daphnia Tox*

*Test abandoned due to unmeasurable concentrations of test material. Same situation inferred for the other, less soluble cat.members.

For the environment, only non-standard acute tests with fish and daphnia were available for one category member, ESBO. A chronic test was started with the most water soluble category member, EODA, but no test substance was measured above the 0.02 mg/l LoD using the water accommodated fraction (WAF) approach. Therefore the test was discontinued. ESBO also had valid algal inhibition data, again read-across to the other category members. Given the high log Kow values for all 4 category members and comparably low water solubility in addition to similar structural features, the read-across was stated to be robust.

Category issues raised during comment/SIAM:
Lack of metabolism data on these chemicals made category justification questionable. However, the common knowledge of fat metabolism by lipases and esterases, low toxicity shown in the repeated-dose toxicity studies, and available potential hazard information on minor metabolites strengthens the justification for grouping these chemicals into the category.

Originally the only algal study (ESBO) was deemed invalid, and given the lack of valid guideline ecotox studies it was recommended that another algal study be conducted with one category member. The algal study was re-evaluated and found to be valid; read-across was then deemed valid.

In addition it was recommended that a further attempt be made towards a chronic daphnia study. Justification for discontinuing the original test was provided and deemed satisfactory.

Differences in available biodegradation tests with one category member indicated a discrepancy in ready vs. non-ready biodegradation status. Justification given and biodegradation status confirmed and subsequently read-across applied.

Generic lessons learned:
- When grouping chemicals together based on common metabolic pathways, include appropriate data or discussion on how these chemicals are related, structurally, functionally, or metabolically.

- Good example of an organic category in which members have similar functional groups and overall structural arrangement and physico-chemical properties, but differences in size/chain length and numbers of functional groups/side chains which seem not to affect its validity. As mentioned read-across is heavily relied upon for the category; similarities in Log K_{ow}, water solubility and structure help to justify this.

Lessons learned specific to the category:
- Health: Provide strong and appropriate justification when metabolism data are not available.

- Environment:
  - The read-across used is simplified for ecotoxicity in that it is essentially qualitative rather than numeric; i.e. effects are defined as only to be seen above water solubility limit. The “continuum” category approach does not apply.
  - The use of QSARs could have further justified the ecotoxicity read-across within the category, by the comparison of QSAR data between members that were read-across.

Case Study #16 (Environment Review Only)
Category Name: Methanolates

SIAM: 22

Category members:

Chemical name	CAS No.
Sodium Methanolate (NaOMe)	124-41-4
Potassium Methanolate (KOMe)	865-33-8

Brief description of category:
Two members have similar physico-chemical properties, rapid reaction in water to give similar degradation products (methanol and sodium/potassium hydroxide), and use patterns. Both NaOH and KOH agreed low hazard for the environment. The primary hazard issue is with methanol toxicity.

Category issues raised during comment/SIAM:
A minor issue was raised regarding the treatment of methanol, the degradation product from both category members. As methanol is a concern for human health (as per its SIDS assessment), this needed to be mentioned, although it did not influence the conclusion for this category.

Generic lessons learned:
- For categories that cover reactive chemicals, the reaction/degradation products must be of a similar nature for each member of the category to be credible.

Lessons learned specific to the category:
In this category the compounds react rapidly with water to form similar compounds and methanol. Methanol is the only degradation product of concern (human health), but did not affect the conclusion for the category. Methanol has its own SIDS assessment.

- Health: Not Reviewed.

- Environment: None

Case Study #17 (Health & Environment Review)

Category Name: Primary Amyl Acetate

SIAM: 22

Category (chemical) members:
Primary Amyl Acetate (Mixed Isomers), mixture composed of:

Chemical name	CASNo.
65% 1-pentyl acetate	628-63-7
35% 2-methyl-1-butyl acetate	624-41-9

Brief description of category (not true category):
Not a category but a mixture composed of two isomers as a result of the reaction process, and supplied as such. Physico-chemical properties, where available, for the two isomers are presented in addition to values for the mixture. Read-across from 1-propyl acetate (CAS No. 109-60-4) and 1-butyl acetate (CAS No.123-86-4) is used to support the acute fish toxicity data generated using Primary Amyl Acetate.

The SIDS document presents data for the mixture, which is a reaction process-derived commercial mixture of two isomers. Additional data for the individual components are presented in support of data for the mixture. The individual components were not sponsored and are not HPV chemicals in the U.S.

Category issues raised during comment/SIAM:
The mixture has no single CAS number.

The assessment was of the composite mixture only, with data from the isomers used as support for the mixture.

The individual isomers are not HPVs in the U.S. Inclusion of the individual isomers as "supporting" information to the mixture does not qualify the individual isomers as having completed the SIDS/SIAM process.

Generic lessons learned:

- In some cases, individual chemicals are HPV only as part of a mixture. Assessment of the mixture does not quality the individual isomers as having gone through the SIDS/SIAM process, if they have been used only for "supporting" or analogue information. If the individual components of the mixture become HPV apart from the mixture, they will need to be sponsored separately.

- Under REACH a challenge will be to convince industry to include all relevant members based on the basic properties excluding use pattern/exposure (and come to different conclusions for different members depending on hazard and use).

Lessons learned specific to the category:

- Health: None

- Environment: None

Case Study #18 (Health & Environment Review)

Category Name: Primary Amyl Alcohol

SIAM: 22

Category (chemical) members:
Primary Amyl Alcohols (Mixed Isomers), mixture composed of:

Chemical name	CASNo.
65% 1-pentyl alcohol	71-41-0
35% 2-methyl-1-butyl alcohol	137-32-6

Brief description of category (not true category):
Not a category but a mixture composed of two isomers as a result of the reaction process, and supplied as such. Physico-chemical properties, where available, for the two isomers are presented in addition to values for the mixture.

The SIDS document presents data for the mixture, which are reaction process-derived commercial mixtures of two isomers in each case. Additional data for the individual components are presented in support of data for the mixture. The individual components were not sponsored and are not HPV chemicals in the U.S.

Category issues raised during comment/SIAM:
The mixture has no single CAS number.

The assessment was of the composite mixture only, with data from the isomers used as support for the mixture.

The individual isomers are not HPVs in the U.S. Inclusion of the individual isomers as "supporting" information to the mixture does not qualify the individual isomers as having completed the SIDS/SIAM process.

Generic lessons learned:

- In some cases, individual chemicals are HPV only as part of a mixture. Assessment of the mixture does not quality the individual isomers as having gone through the SIDS/SIAM process, if they have been used only for "supporting" or analogue information. If the individual components of the mixture become HPV apart from the mixture, they will need to be sponsored separately.

- Under REACH a challenge will be to convince industry to include all relevant members based on the basic properties excluding use pattern/exposure (and come to different conclusions for different members depending on hazard and use).

Lessons learned specific to the category:

- Health: None

- Environment: None

Case Study # 19 (Environment Review Only)

Category Name: Bicarbonate Special

SIAM: 22

Category members:

Chemical name	CAS No.
Sodium Bicarbonate	144-55-8
Sodium Carbonate	497-19-8
Ammonium Bicarbonate	1066-33-7

Brief description of chemicals (not a true category):
Not a true category in that commercial material is a reaction mixture of the 3 components [$NaHCO_3$ (81%), Na_2CO_3 (13%), NH_4HCO_3 (3%)]. Separate SIDS dossiers are referred to for each component ($NaHCO_3$ and Na_2CO_3 in SIAM 15; NH_4HCO_3 separately in SIAM 22) hence the category approach is applied.

Similarities:
- all 3 are stable solids except NH_4HCO_3 which decomposes with heat
- all 3 components dissociate to Na^+/NH_4^+ and HCO^{2-}/CO_3^{2-} in aqueous solution
- bicarbonate and carbonate ion can be considered as interchangeable in aqueous solution with the relative concentrations of each being pH dependent, regardless of the form released
- all 3 have similarly low toxicological profiles; exceptions are NH_3 corrosivity and sodium carbonate is harmful by inhalation
- only NH_3/NH_4^+ cation cause concern for the environment

Category issues raised during comment/SIAM:
The use of a category approach was questioned because all of the proposed category members have been assessed separately. The category approach was upheld as it was felt it was the best approach to this mixture (the result of a complex reaction and not a formulation).

Generic lessons learned:
- For categories including ionisable compounds, water solubility and dissociation behaviour in aqueous solution should be considered. These characteristics should be similar or follow a predictable trend among category members. In this case, the (effectively) complete dissociation of all 3 components reinforces the category approach.

- For categories including ionisable compounds, pH effect on behaviour in aqueous solution must be considered.

Lessons learned specific to the category:

- Health: Not Reviewed

- Environment:
    - Read-across not important in this case - NH_3/NH_4^+ cation assessed separately to other components. HCO^{2-}/CO_3^{2-} essentially the same in solution (depend on pH).

- In this case, the (effectively) complete dissociation of all 3 components reinforces the category approach.
- In this case the bicarbonate and carbonate anions are essentially the same (concentrations governed by solution pH), which strengthens the category approach.

Case Study #20 (Environment Review Only)

Category Name: Oxo Alcohols C9 to C13

SIAM: 22

Category members:

Chemical name	CAS No.
Alcohols C8-C10-iso, C9 rich	68526-84-1
Isononyl alcohol	27458-94-2
Alcohols C9-C11-iso, C10 rich	68526-85-2
Isodecyl alcohol	25339-17-7
2-Propylheptan-1-ol	10042-59-8
Alcohols C11-C14-iso, C13 rich	68526-86-3
Isotridecan-1-ol	27458-92-0

Brief description of category:
Straight chain alkyl alcohols with one methyl or ethyl side chain except one member (propyl side chain); total number of carbon atoms (inc. side groups) C9 – 13; a small "continuum" category. All, except 2-propylheptan-1-ol, are manufactured in the same way and the category is justified by 4 factors: similarity in chemical structure, physical-chemical properties, environmental fate and toxicological mode of action.

Category issues raised during comment/SIAM:
The category member with the propyl side chain was highlighted as a possible outlier to the category. However, the 4 factors considered in the category justification all support its inclusion.

Generic lessons learned:
- For this "continuum" category the approach taken was to divide the assessment discussion into a separate paragraph describing specific endpoints for each member for the (eco)toxicological sections. Summaries including graphical illustrations of the trends in data (e.g. linearly increasing aquatic toxicity with chain length) are very useful. This approach worked well as the category is both small (7 substances) and for the most part data rich.

Lessons learned specific to the category:
- Health: Not Reviewed

- Environment: The formation of sub-groups within a "continuum" category can be elaborated with consideration of the relevant endpoint(s). In the HPV programme 2 category members (C13) exhibited higher ecotoxicity than the other members and so formed a sub-group for the conclusions in the assessment.

Read-across Example: Cd & Cd Compounds Human Health

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7 July 2006

Read-across example Cd & Cd compounds human health

The ESR Cd-RA (September 2004) assessed risks associated with exposure to cadmium metal and cadmium oxide. Data with other Cadmium compounds were used as supporting data ('*data on cadmium compounds are included when no (not enough) information on the effects of CdO/Cd metal was available and when the studies using cadmium compounds were mechanistically relevant*').

Available data:

Compound	Cadmium metal	Cadmium oxide fumes	Cadmium oxide(dust)	Cadmium chloride	Cadmium sulphide Other Cd compounds
Solubility (mg/L)	✓/- Insoluble (0,05 mg/1 at pH 10,5 a curve in function of pH and hardness		✓/- Insoluble	✓/- soluble	✓/- soluble
Spherical diameter	18 µm +/- 13.3 µm		0.55 µm		
Oral absorption	- <5%		- <5%	✓ 2%	✓/- 0.5-12%
Dermal Absorption	- <1%		- <1%	✓/- 1%	-
Inhalation absorption	- 10-30%	✓ 25-50%	✓ 10-30%	-	-
Acute inhalation toxicity (mg/m³)	- T+,R26	✓/- 25-30 (15 min.) T+,R26	- T+,R26	-	✓ **28.4** (Cd iron dust, 4 hours)
Acute oral toxicity (mg/kg BW)	✓/- LD50 2330		✓/- LD50 :72 -296	✓/- LD50: 63-88	✓/- LD50: 590-1125 (Cd stearate)
Acute dermal toxicity (mg/kg BW)	-		-	-	-
Irritation respiratory tract	- no classification proposal	- no classification proposal	- no classification proposal		
Irritation: eye, skin	- no classification proposal	- no classification proposal	- no classification proposal	-	-
Sensitisation	- no classification proposal	- no classification proposal	- no classification proposal	-	-
Mutagenicity	- Muta Cat 3; R68	✓/- Muta Cat 3; R68	✓/- Muta Cat 3; R68	✓	✓
Carcinogenicity	- Carc Cat 2; R45	✓ Carc Cat 2; R45	✓ Carc Cat 2; R45	✓	- Carc Cat 2; R45
Repro: fertility	- Repr Cat 3; R62	- Repr Cat 3; R62	✓ NOAEL 0.1 mg/m³ Repr Cat 3; R62	✓ NOEL: 1 mg/kg/day	-
Repro: development	- Repr Cat 3; R63	✓/- Repr Cat 3; R63	✓/- Repr Cat 3; R63		
RDT lung	-	✓ LOAEL 3 µg/g creat	✓ NOEL 0.01 mg/m³		
RDT bone and kidney	- LOAEL 2 µg/g creat R48/23/25	✓ LOAEL 2 µg/g creat R48/23/25	- LOAEL 2 µg/g creat R48/23/25		-

✓: data available of good quality ✓/-: data available but of limited to poor quality -: no data available

Read-across was applied to derive classification proposals and assess risks for the substances/endpoints shaded in yellow:

-for oral absorption of CdO, Cd metal
-for inhalation absorption of Cd metal
-for dermal absorption of Cd metal, CdO
-for acute inhalation toxicity of CdO dust, Cd metal
-for mutagenicity of Cd metal, CdO
-for carcinogenicity of Cd metal
-for reproductive toxicity (fertility) of Cd metal, CdO
-for reproductive toxicity (development) of Cd metal
-for repeated dose toxicity of Cd metal

Toxicokinetics

Digestive absorption rates varying between 0.5 and 12% (on the average 2%) have been reported according to the animal species and the chemical form of cadmium. The higher values have been reported for monkeys and large animals compared to rodents. In small animals, absorption in the range of 1 to 2% is commonly reported (CEC, 1978; Nordberg, 1985).

Little is known about the mechanism of uptake of the various forms of Cd and the transport across the epithelial cells in the intestine.

It seemed reasonable to assume that gastrointestinal absorption of CdO is not significantly different from that of other Cd compounds, mainly because of the high solubility of CdO (and probably Cd metal) in gastric juice (94 %). Therefore, data from studies conducted with other Cd compounds were judged relevant for assessing the gastro-intestinal absorption of CdO/Cd metal in this RAR. Overall, it was considered that a large proportion of ingested Cd (including from CdO) is eliminated in the faeces and that only a few percent (maximum 5%) is absorbed via the gastrointestinal tract.

After inhalation, the alveolar absorption rate of Cd from CdO varies depending on the type of exposure (fumes>dust; intra-tracheal>inhalation). It is a slow process that continues for many weeks after a single inhalation exposure (Nordberg et al, 1985). Absorption rates after inhalation of CdO derived from animal studies range from 50 % (fumes) to 30 % (dust, depending on particle size).In humans, figures of **10-30 %** of absorption rate according to particle size are derived for CdO dust (Task Group 1973).

Although specific data for Cd metal dust are not available, it appeared reasonable to assume that it does not differ greatly from CdO.

Acute inhalation toxicity

The acute pulmonary toxicity of cadmium seems to depend on the chemical and physical form of the administered compound, and therefore the question of the validity of an extrapolation to CdO of results obtained with other compounds had to be considered in the Cd RA.

Different Cd compounds tested in rats were not equivalent with respect to toxicity: CdO fumes and Cd carbonate appeared to be more toxic than two cadmium pigments, "cadmium red" (Cd: 69.9%, Se: 16.4%, S: 13.2%) and "cadmium yellow" (Cd: 77.4%, S: 21.7%, Zn: 0.28%, Se: 0.27%) (Rusch et al, 1986). Difference in toxicity was attributed to an *increased retention and greater absorption* for the more soluble compounds (carbonate and oxide fumes) compared to the highly insoluble cadmium pigments which have a greater mucociliary clearance.

The CT_{50} for cadmium oxide dust would be about three to four times the values for cadmium oxide fumes (Friberg, 1950), and this would be explained by a much longer retention of CdO dust in the lung (Oberdörster et al.,1992). The retardation of the clearance of the CdO oxide particles may be due to a slower solubilisation of the particles, compared to that of the fine and very porous fume particles (Oberdörster, 1992).

On the other hand, some authors warned of predicting the behaviour of compounds in complex biological systems by their chemical solubility alone (Glaser et al., 1986). For example, based on the water solubility of CdO and CdS that are very low compared to the highly soluble $CdCl_2$, one might predict a higher bioavailability of $CdCl_2$ *in vivo*.

The pulmonary effects of water insoluble cadmium oxide (dust, fumes) were compared with those induced by water-soluble cadmium chloride in groups of rats exposed by inhalation by Oberdörster et al. (1987). Small CdO particles and $CdCl_2$ were equally toxic. Authors concluded that acute effects of Cd compounds in the lung cannot only be predicted from their water solubility

(Oberdörster et al, 1987: the *in vivo* solubility in the lung after inhalation exposure is very high for CdO (Oberdörster and Cox, 1990).

Inhaled CdO appeared to be even more damaging to the lung than $CdCl_2$ in the experiment conducted by Grose et al.. (1987) who compared the effects of aerosols of both compounds on the pulmonary biochemistry and histology in rats and rabbits. Authors concluded that because of the more acute response of the lung to CdO compared to $CdCl_2$, extrapolation of $CdCl_2$ effects to potential CdO effects could be scientifically vulnerable (Grose et al, 1987).

For Cadmium metal, no data were available and read-across was done from the CdO data as exposure is mainly to CdO.

Sensitisation

No test results with cadmium (oxide) as test substance were submitted. A single test with a soluble cadmium salt ($CdCl_2$) was located in animals (with negative result but insufficient information to document the test conditions). No read-across was done, no proposal for classification was made.
No study specifically using cadmium metal was located. Since inhalation exposure to cadmium metal dust is very unlikely in occupational settings, this absence of information is not deemed critical.

Repeated dose toxicity

Lung: Long-term inhalation exposure of experimental animals to CdO results in similar effects as seen upon acute exposures, i.e. pneumonia accompanied by histopathologic alterations and changes in the cellular and enzymatic composition of the broncho-alveolar fluid. Identified NOAELs are: 0.025 mg CdO/m^3 in F344/N rats exposed for 13 weeks and 0.01 mg Cd/m^3 in hamsters exposed for 16 months.
From the available human studies, a LOAEL of 3.1 µg Cd/l (Cd-U) was derived taking into consideration that this value is for CdO fumes and may not necessarily apply to CdO or Cd metal dust.

Bone: Because most of the experimental studies were designed to explore the pathogenesis of Itai-Itai disease and because animals were generally exposed during a relatively short period with relatively high doses of Cd water soluble compounds, they do not allow to derive a robust NOAEL relevant for humans exposed chronically to low doses via the diet or by inhalation.

The kidney is another target organ for cadmium (not specifically Cd metal) toxicity following repeated exposure by the inhalation and oral routes. Numerous studies in rats, mice, rhesus monkeys and rabbits have indicated that exposure to cadmium compounds administered orally or by inhalation causes kidney damage including increase or decrease of relative kidney weight, histological (necrosis of the proximal tubules, interstitial renal fibrosis) and functional changes (reduced glomerular filtration rate, proteinuria). On the basis of the most recent human studies conducted in Europe, a LOAEL of 2 µg/g creatinine is considered for the risk characterisation.

Genotoxicity

No in vitro study using cadmium metal was identified. Bacterial tests with cadmium oxide yielded negative results. Most of the located in vitro studies used water-soluble cadmium chloride. While, water solubility does not necessarily reflect in vivo solubility, it was assumed that Cd/CdO will to some extent be solubilised in vivo, especially in the lung, and data obtained with soluble Cd

compounds were considered relevant to assess the possible genotoxic potential (hazard) of cadmium oxide.
Although a clear and consistent pattern of action remains to be determined (direct damage by interacting with the chromatin to generate strand breaks, cross-links or structural alterations in DNA, and/or indirectly, by depleting antioxidant levels and thereby increasing intracellular hydrogen peroxide and other oxidants, and/or by interacting at metal-binding sites of proteins involved in transcription), it was concluded that cadmium metal and oxide can exert a genotoxic potential in vitro in consideration of several genotoxic effects reported with water soluble cadmium compounds.

Carcinogenicity

An unequivocal relationship between Cd exposure and lung cancer incidence was demonstrated in chronic inhalation studies in Wistar rats exposed to $CdCl_2$, CdO fumes and CdO dust. In two inhalation studies in rats, malignant lung tumours were produced by cadmium oxide dust and fumes at low levels of exposure for short duration. The lowest dose to produce carcinogenic effects was 30 µg Cd/m³ as cadmium oxide dust as well as cadmium oxide fumes. Groups of rats exposed to cadmium oxide fumes (10 µg CdO/m^3, 30 µg CdO/m^3) had significantly lower lung tumour incidences than those seen in groups exposed to cadmium oxide dust using the same the exposures modalities. However, these animals had only about half the cadmium content in their lungs compared to animals exposed to the same concentration of CdO dust over the same period, attributed to a lower pulmonary deposition of the chain-like electric arc-generated fume particles (IARC 1993 reporting Oberdörster & Cox, 1989).
Mice exposed to equivalent levels of cadmium oxide, had only marginally significant elevations in lung cancer, but the rate of lung cancers in control mice was variable and elevated. No evidence for lung carcinogenicity was found in hamsters, possibly due to lung damage and subsequent decreased survival at high doses.
Interspecies but also interstrain differences seem to play a role in the sensitivity to Cd-induced carcinogenesis. Intratracheal instillation of cadmium oxide caused no increase in lung tumours in rats, but did increase the incidence of mammary fibroadenomas. Cadmium oxide is a carcinogen in rats when injected locally at the site of injection. Cadmium metal powder is a carcinogen in rats when injected intramuscularly forming malignant tumours.

Only one study reported an increase in cancer upon oral exposure to soluble cadmium compounds; no data were located for cadmium metal or cadmium oxide.

Toxicity for reproduction

Fertility

By the oral route, no animal study specifically using cadmium oxide or metal has been identified. The lowest concentration of cadmium reported to affect fertility in rats was 10 mg Cd/kg/day (number of copulations and pregnancies, number of implants and fetuses were decreased) when males and females rats were both treated with Cd water soluble compounds.

Inhalation exposure to cadmium oxide at a concentration of 1 mg/m^3 (for more than 10 weeks) has been associated with an increase in oestrous cycle length and reduction of the number of spermatids in testis in rats.

The overall NOAEL is 0.1 mg CdO/m³ (about 0.09 mg Cd/m³). No study specifically using cadmium metal by inhalation was located.

Developmental toxicity

No study specifically using cadmium oxide or cadmium metal by the oral route was located. Neurobehavioural effects or changes in electrophysiological parameters were reported to occur at doses that did not induce maternal toxicity. Lowest dose reported to generate behavioural changes in pups is 0.04 mg Cd/kg/day (LOAEL) (Baranski et al., 1983). Significance of these changes and underlying mechanisms for the observed effects on behavioural endpoints are not completely elucidated yet; some authors suggested that the toxic effects might be mediated by placental toxicity or by interference with the normal fœtal metabolism of Zn and/or Cu. Because the oral bioavailability of CdO and Cd metal is not fundamentally different from the compounds tested, it can reasonably be considered that these observations can be extended to Cd metal and CdO by the oral route.

Inhalation route: No study using cadmium metal was located. Some studies using cadmium oxide and investigating foetal body weight, malformations or neurobehavioural effects of inhaled cadmium have been identified (Baranski, 1984; Baranski, 1985; NTP Report 1995). Neurobehavioural changes were reported in young rats from dams exposed to CdO (0.02 mg Cd/m^3 or about) in an apparently single experiment but these observations should be confirmed in an independent study.

Read-across Example: Cu & Cu Compounds Human Health

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Read-across example Cu & Cu compounds human health

Read across has been used under a select set of circumstances in the ongoing Voluntary Copper Risk Assessment. For purposes of testing derogation and acute toxicity classification, read across has been based upon evaluation of physical properties (e.g. particle size in dustiness testing), water solubility and oral absorption.
Cu-VRA substances: copper (metal), copper (I)oxide, copper(II)oxide, copper sulphate pentahydrate, copper oxychloride

Data available

Compound	Cuprous (I) oxide	Copper (II) oxide	Copper sulphate	Copper powder	Copper oxychloride
Solubility (mg/L)	✓ > 6.39	✓ <0.39	✓ 266000	✓ insoluble	✓ > 1.19
D50 (µm) MMAD of marketed material D50 (µm) and dustiness of airborne fraction (mg/g)	✓ 9.9 3.3 7.07	✓ 60.7 32.5 363.71	✓ 90.3 220.4 48.75	✓ 71.7 129 45.57	✓ 12.2 2.3 33.36
Oral absorption	✓/-	✓/-	✓ function of the dose	✓/-	✓/-
Dermal Absorption	✓/- 0.3%wet 0.03%dry	✓/- 0.3%wet 0.03%dry	✓/- 0.3%wet 0.03%dry	✓/- 0.3%wet 0.03%dry	✓/- 0.3%wet 0.03%dry
Inhalation absorption	-	-	-	-	-
Acute inhalation toxicity (mg/L)	✓ LC50 3.34-5.36	-	-	-	✓ LC50 4.74->11.4
Acute oral toxicity (mg/kgBW)	✓ LD50 200-1340	✓ LD50 >2500	✓ LD50 482-666	-	✓ LD50 300-1860
Acute dermal toxicity (mg/kgBW)	✓ LD50 >2000	✓ LD50 >2000	✓ LD50 >2000	✓ LD50 >2000	✓ LD50 >2000
Irritation-eye	✓ R36	✓ no effects	✓ R41	✓ no effects	✓ no effects
Irritation-skin	✓ no effects	✓ no effects	✓ no effects	✓ no effects	✓ no effects
Skin sensitisation	✓ no effects	✓ no persistent effects	✓ no persistent effects	✓ no effects	✓ no effects

✓: data available of good quality
✓/-: data available but of limited to poor quality
-: no data available

Read-across was applied to derive classification proposals and assess risks for the substances/endpoints shaded in yellow:
-for acute inhalation toxicity for Cu(II)O, Cu-powder and CuS04.5H2O
-for acute oral toxicity for Cu-powder
-for mutagenicity
-for carcinogenicity
-for reproductive toxicity
-for repeated dose toxicity

Properties used to read-across from compounds with data to compounds without data or with no good quality data:
-solubility in water
-oral absorption
-dustiness information and particle size distribution

Acute oral toxicity for Cu-powder

Copper powder

Read-across was based on solubility and available acute oral toxicity information for the other compounds:

LD50 of the least soluble compound Cu(II)O: >2500 mg/kgBW; no classification.

The more soluble compounds all classify as harmful.

In view of the poor solubility of copper powder, it is considered appropriate to read-across from Cu(II)O.

Conclusion: copper powder no classified

Acute inhalation toxicity for Cu(II)O, Cu-powder and CuS04.5H2O (still under discussion; based on proposal prepared by EBRC)

Copper (II) oxide

Read-across was based on the following information:

-Copper (II) oxide has a low oral toxicity (LD_{50} > 2000 mg/kg bw), and

-a low water solubility (< 0.39 mg/L). Although toxicokinetic data in laboratory animals are not available for this compound, investigations in feeding trials with cattle, pigs and chicken indicate a very limited bioavailability compared to other copper compounds.

-Laboratory investigations on the propensity to become airborne suggest a moderate mobility (i.e., total dustiness measured at 364 mg/g). The particle size distribution of airborne matter shows a mass median aerodynamic diameter of more than 60 microns. Using the MPPD model, the deposition pattern in humans (extrathoracic 49%, tracheobronchial 0.9%, alveolar fraction 1.1%) and rat was derived (total deposition of 34%, with 33% deposited in the extrathoracic region, 0.4 % in the tracheobronchial region, and 0.4% in the alveolar fraction).

Summary:

- Copper (II) oxide has a moderate (but not negligible) tendency to become airborne,

-based on particle size considerations (MMAD > 60 µm), more than 95% of the material deposited in the respiratory tract will be translocated to the GI tract shortly after inhalation, so that the acute toxicity will be determined by that of the oral route,

- Copper (II) oxide is assumed to have a low bioavailability, based on water solubility and on indicative data from animal feeding studies,

- it has an established low oral toxicity (LD_{50} > 2000 mg/kg bw).

Conclusion:

the inhalation hazard of copper (II) oxide is very low and it was therefore proposed not to classify copper (II) oxide for acute inhalation toxicity.

Copper powder

-Copper powder has not been tested for acute oral toxicity, but by way of read-across from data on copper (II) oxide and its extremely low water solubility (<< 1 mg/L) has been assigned a low oral toxicity, not requiring classification.
-In analogy with copper (II) oxide, a very limited bioavailability compared to other copper compounds is similarly assumed.
-Laboratory investigations on the propensity to become airborne suggest a very low mobility (i.e., total dustiness measured at 46 mg/g). The particle size distribution of airborne matter shows a mass median aerodynamic diameter of more than 70 microns, based upon which the following deposition pattern in humans was derived using the MPPD model: extrathoracic 49%, tracheobronchial 0.9%, alveolar fraction 1.2%. The corresponding prediction for the rat is 34% total deposition, with 33% deposited in the extrathoracic region, 0.4 % in the tracheobronchial region, and 0.4% in the alveolar fraction.

Summary:
- Copper powder has a negligible tendency to become airborne (i.e., less than 5%),
- based on particle size considerations (MMAD > 70 µm), more than 95% of the material deposited in the respiratory tract will be translocated to the GI tract shortly after inhalation, so that the acute toxicity will be determined by that of the oral route,
- Copper powder, in analogy to copper (II) oxide and based on the extremely low water solubility, is assumed to have a low bioavailability,
- Copper powder is assumed to have a low oral toxicity (LD_{50} > 2000 mg/kg bw) by read-across from copper (II) oxide.

Conclusion:
the inhalation hazard of copper powder is very low, and it was therefore proposed not to classify copper powder for acute inhalation toxicity.

Copper sulphate pentahydrate

-Copper sulphate pentahydrate has been tested several times for acute oral toxicity, with resulting LD_{50} values in the rage of 480 – 960 mg/kg bw, resulting in a classification as “harmful via ingestion”. Similarly, the anhydrous form has yielded an LD_{50} of 300 mg/kg bw, in line with the slightly elevated relative copper content compared to the hydrate. In contrast, no data is available on acute inhalation toxicity of copper sulphate pentahydrate.
- Copper sulphate pentahydrate has a low relative copper content (25%, compared for example to copper powder (100%), copper (I) oxide (89%) and copper (II) oxide (80%)
- the oral bioavailability of copper sulphate in comparison to other copper compounds at dose levels relevant for acute toxicity testing has been assessed as being similar, i.e. 11-13%

- laboratory investigations on the propensity to become airborne suggest a negligible mobility (i.e., total dustiness measured at 49 mg/g). The particle size distribution of airborne matter shows a mass median aerodynamic diameter of more than 90 microns, based upon which the following deposition pattern in humans was derived using the MPPD model: extrathoracic 49%, tracheobronchial 1.0%, alveolar fraction 1.2%. The corresponding inhalation deposition prediction for the rat is 34% total deposition, with 33% deposited in the extrathoracic region, 0.4 % in the tracheobronchial region, and 0.4% in the alveolar fraction
- technical considerations render inhalation toxicity testing of copper sulphate pentahydrate technically unfeasible: the d50 physical particle size has been determined at 220 µm.
- without manipulation of particle size (i.e. by micronisation), more than 95% of inhalable copper sulphate pentahydrate would be deposited in the extrathoracic region, and subsequently and rapidly translocated to the GI tract; however, only approx. 5% of the material present in commercial grades of this substance can at all become airborne
- even if a mechanical micronisation procedure down to particle sizes in the region of those tested for other copper compounds (i.e., copper (I) oxide and copper oxychloride) would be employed, the thus generated material would render a similar deposition pattern in the respiratory tract, again with predominant translocation to the GI tract; however, based on the rel. copper content of only 25%, the inhalation of particles of the same size range as those already tested would deliver only one third to one quarter of the amount of copper by comparison
- given that copper (I) oxide and copper oxychloride have been tested in multiple assays, and have yielded “borderline” results (i.e, some LC_{50} value above 5 mg/L, some below), then it can not be expected that copper sulphate pentahydrate can cause any toxicity beyond that observed for these two compounds; instead, less copper is expected to become systemically available
- toxicity other than systemically available copper cations is not to be expected: any copper sulphate will deposit almost exclusively in the ET fraction, from where systemic absorption is unlikely to occur; absorption from this region is commonly extrapolated from dermal absorption data, which is known to be low (0.3%).
- given that copper sulphate is only moderately irritant and not corrosive, any direct local action is also not anticipated
- the residual very low amounts of copper sulphate that may reach the tracheobronchial based on the predictions set forth below may at first glance be subject to dissolution; however, any dissolution is effectively counteracted by the high carbonate content (50 mM) of airway fluids, so that any material that becomes available would be rendered insoluble as copper carbonate

Conclusion:
the inhalation hazard of copper sulphate pentahydrate is very low; and it was therefore proposed not to classify copper sulphate pentahydrate for acute inhalation toxicity

Mutagenicity, Carcinogenicity, Reproductive toxicity, RDT

Toxicity data are available for CuSO4.5H2O and no data is available for the other compounds.

Classification proposals and risk analysis for the non tested compounds are based on the conclusions for CuSO4.5H2O assuming that copper in the GIT will occur, at least in part, in the ionic form and will therefore be available for absorption. Whilst bioavailability data exist for several copper substances in addition to copper sulphate (copper (I) oxide, copper (II) oxide and copper oxychloride), the database is limited in terms of quality and/or relevance to human risk assessment. Further, no bioavailability data are currently available for copper powder. In the absence of reliable bioavailability data for all copper substances, as a worst case situation, it was considered appropriate to read-across from copper sulphate, on the basis that this is the most soluble and apparently bioavailable copper substance covered here, and the most well-characterised with respect to toxicokinetics and repeated dose toxicity.

Read-across Example: Ni & Ni Compounds

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7 July 2006

READ-ACROSS: Example Ni and Ni-compounds
Basis: Data collected for the EU-RA and EU classification and labeling of Ni and Ni-compounds

The document provides principles on how to apply read-across and gives examples for certain endpoints.

I. Background: In its simplest form, read-across is an extrapolation of known data from one substance to another substance on the basis of assumptions leading to a conclusion that the two substances will cause similar biological responses. This basic premise of science is used in the regulatory setting to reduce the need for animal testing. The most detailed embodiment of the concept of read-across has occurred in the production of Qualitative Structure Activity Relationship (QSAR) models, developed for organic compounds and based on the presence of active groups within the organic molecule that are capable of eliciting certain biological effects. For metal substances, it is very important to understand that the simple presence of a metal in a substance does not necessarily impart to that substance the biological properties of the metal ion. It is the bioavailability of the metal ion (or a redox form of this ion) at target sites that needs to be assessed for the read-across of metal substances to be accurate.

In general, regulatory read-across seeks to extrapolate the biological response data for required toxicity testing endpoints. These endpoints can be summarized as follows:

- Acute Toxicity – Inhalation, Oral, Dermal
- Irritation – Eye, Dermal
- Sensitization – Skin, Respiratory
- Repeated Dose Toxicity – Inhalation, Oral, Dermal
- Mutagenicity
- Carcinogenicity – Inhalation, Oral, Dermal
- Reproductive – Developmental Toxicity, Gonadal Toxicity (Fertility)

For the purposes of regulatory read-across, toxicity categories can be defined that will aid in reducing testing requirements for a group of similar substances. Specifically, for inorganic substances and many organic substances four categories can be developed:

1. Systemic threshold toxicity
2. Systemic non-threshold toxicity
3. Contact threshold toxicity
4. Contact non-threshold toxicity

The major difference between the **contact and systemic toxicity categories** lies in the manner in which the substance is made bioavailable to the target tissue.

- In the case of dermal, eye, or inhalation toxicity, bioavailability is dictated by a series of local events that will enable the metal ion in the substance to interact with target cells and organelles in the tissue of contact. These events could include phagocytosis, passive and active membrane transport, and redox changes.
- In the case of systemic toxicity, all of these local events are equally important. However, prior to consideration of whether a substance will have similar biological activity at a systemic site of action, one needs to consider whether the substance will be absorbed from the respiratory tract by inhalation, from the skin, or from the gastrointestinal tract after oral exposure and transported to the site of action. Therefore, in the case of systemic toxicity,

oral exposure nearly always results in the highest systemic exposure of a substance and hence may often be the route of exposure used to determine if the criteria for grouping and extrapolation is appropriate.

The same issues hold for **systemic and contact non-threshold toxicities** (*e.g.*, most commonly mutagenicity and carcinogenicity) with the exception that there is no safe level of exposure associated with zero risk of causing the toxicity in question.

While most of the issues surrounding the concept of bioavailability at target sites is self evident, it is still essential to validate the grouping criteria, by demonstrating an equivalency of bioavailability between the substances of a proposed grouping. Doing so constitutes the validation of the grouping criteria stipulated by the OECD and TAPIR Annex 9 as essential components of the read-across process. In fact, there are a number of common validation principles across all of these approaches that need to be addressed to a greater or lesser extent in order for the output to be accepted (TAPIR Annex 9). These include:

- transparency - both of method and data;
- definition of applicability domain. This domain refers to those chemicals that the approach can make reliable predictions for;
- assumption, or recognition of a common mode of action or mechanistic understanding;
- validation of model/output.

II. Extrapolating Threshold Based Toxicities

For regulatory purposes, **threshold based toxicities** include all the endpoints mentioned in section I, except mutagenicity and carcinogenicity (and in some cases respiratory sensitization).

- In the case of most systemic threshold-based toxicities, oral exposure provides the highest potential for systemic exposure levels.
- In the case of threshold-based contact toxicities dermal, eye, and inhalation routes of exposure must be evaluated.

In this framework, a scientifically valid grouping and read-across (from a "reference substance" to the "substance under evaluation") for threshold based systemic toxicities is possible.

1. No read across is needed
   There is data on systemic effects seen with that specific substance under evaluation.

2. Best read across
   - Obtain toxicokinetics data on NOAEL blood levels of a reference substance from the group with a known toxicity profile
   - Compare those levels to the blood levels of the substance under evaluation exposed at its Maximum Tolerated Dose (MTD).

   - If the blood levels of the substance under evaluation are below those corresponding to the NOAEL value for the reference substance, do not classify.
   - If values are higher, classify and use the NOAEL of the reference substance for risk characterization.

3. Limited read across No toxicokinetic data are available.
   - Collect data on oral absorption and oral toxicity (MTD) for the reference and the substance

under evaluation. The balance between oral toxicity and absorption will determine what is the highest absorbed level possible for the substance under evaluation.
- Compare these data.
- Based on expert judgment, decide whether to classify or not.

4. Minimal read across No data on toxicokinetics or oral absorption are available.
   - Generate data on bioaccessibility (dissolution of the substance under evaluation in biologically relevant solutions (*e.g*., lung lavage fluid, gastric juice/intestinal juice, synthetic sweat).
   - Conduct side by side experiments with the substance under evaluation and if possible with more than one reference substance(s). These assays can be used to generate quick and relatively inexpensive data on a large number of substances.

   However, this approach needs to be validated by using several reference substances for which some correlation between bioaccessibility in gastric fluid and oral absorption exists.

5. Not acceptable read across - No data on toxicokinetics, oral absorption, or bioaccessibility are available. Only physical/chemical data exist.
   - This can ONLY be used for read across if the physical/chemical nature of the substance under evaluation is so similar to that of the reference substance, that bioaccessibility and oral absorption are predicted to be almost identical.

III. Test waiving

If for a given substance under evaluation, no data are available for steps 1-4, and the producer does not want to undertake the minimal testing (step 4) or more thorough testing (step 2-3), then the default classification should follow the worst case substance in the proposed grouping category. Such a default classification would represent therefore the most conservative option for default classification.

IV. EXAMPLE: REPRODUCTIVE EFFECTS AND NICKEL SUBSTANCES: CLASSIFICATION AND RISK CHARACTERIZATION.

Data available for classification and risk characterization.

Data were available from one- and two-generation reproductive studies in rats exposed orally to nickel chloride and nickel sulphate studies. The NOAEL for developmental effects seen in rats exposed orally to nickel sulphate equates to 1.1 mg Ni/kg/day. Based on these data, nickel sulphate and chloride were classified as category 2 developmental toxicants.

How to "read across" to other nickel substances?

The determinant of a developmental reproductive effect is the bioavailability of the nickel ion to systemic circulation and ultimately the delivery to the conceptus of pregnant rats. Bioavailability of nickel ion will be influenced by oral absorption.

Read across from nickel sulphate and chloride data to another nickel substance.

1. No read across is needed – There is data on reproductive effects seen with that specific nickel substance.
   Examples: nickel chloride hexahydrate and nickel sulphate hexahydrate.

2. Best read across
   - Obtain toxicokinetics data on blood nickel levels after oral exposure to 1.1. mg Ni/kg of

nickel sulphate hexahydrate (reference substance).
- Measure the same parameters for the other nickel substance under evaluation at the MTD and compare values.

- If the blood levels of the nickel substance under evaluation are below those corresponding to the NOAEL value for nickel sulphate ➔ do not classify.
- If values are higher ➔ classify and use 1.1. mg Ni/kg for risk characterization.

3. Limited read across – No toxicokinetic data are available.
   - Collect data on oral absorption of the nickel substance under evaluation as well as oral toxicity
   - Compare to the values for the reference substance (nickel sulphate hexahydrate).

   Example: read across by the TC C&L for nickel metal as regards to reproductive toxicity, given the absence of toxicokinetic data. Nickel metal had 100-fold lower oral absorption than nickel sulphate, while the oral LD_{50} was over 30-fold higher. The high equivalent oral exposure levels in humans that would be needed to absorb sufficient nickel ion to cause an adverse effect were not considered to be realistic. Expert judgment deemed that nickel metal should not be classified as a reproductive toxicant.

4. Minimal read across – This approach was not used for nickel compounds. However, validation of such an approach could be done by:
   - looking at the oral absorption for some substances that have bioaccessibility values in the middle of the range between sulphate (category 2) and metal (no classification) and
   - making sure that the bioaccessibility value corresponding to the threshold between classification and no classification is well defined (Minimal validation could include comparison of oral absorption and bioaccessibility)

5. Not acceptable read across – No data on toxicokinetics, oral absorption, or bioaccessibility are available. Only data on water solubility exist.
   This can ONLY be used for read across if the water solubility and nature of the nickel substances is so similar to that of the reference substance, that bioaccessibility and oral absorption are predicted to be almost identical.
   For example, read across from nickel sulphate to nickel chloride and nitrate. Both are strong salts that will be expected to be ionized and dissolved completely in the gastric environment.

V. Extrapolating Non-Threshold Based Toxicities

For regulatory purposes, non-threshold based toxicities typically refer to mutagenicity and carcinogenicity. As in the case of systemic threshold based toxicities, oral exposure provides the highest potential for systemic exposure levels. In the particular case of non-threshold based contact toxicities, the inhalation route of exposure must be evaluated and expert judgment should be applied to the need for testing the dermal and eye routes of exposure (*i.e.*, these would be expected to be done infrequently).

VI. How to read across for carcinogenicity?

The most important issue to be able to read across for carcinogenicity classification and risk characterization is to understand the mode of action of the carcinogenic substance. Some important components of mode of action are:

1. local or systemic nature of the cancer effects,
2. mutagenic (genotoxic) or non genotoxic mechanisms for cancer induction,
3. factors that influence bioavailability of metal ion at critical cellular sites.

Complete information on mode of action is not likely to be available for any metal substance and, in fact, for few compounds at all. Therefore, this is one of the most difficult endpoints to consider read across for.

Some examples of differences in components of the mode of action for cancer associated with metal/metalloid substances are as follows:

- systemic effects for arsenic *via* inhalation but only local (respiratory) effects for nickel substances;
- difficulty to draw a universal relation between water solubility and bioavailability
    - water solubility of substances is directly related to bioavailability of cadmium (II) (for tumour induction) from cadmium compounds,
    - water solubility is inversely related to bioavailability of Cr (VI) from chromium compounds (for tumor induction) after inhalation.
    - The relationship between water solubility and bioavailability of nickel (II) at target sites may be even more complex for nickel, with compounds of intermediate solubility having the highest bioavailability.
- Both mutagenic and non genotoxic effects have been postulated to be part of mode of action of most metals.

In the process of assessing weight of evidence for carcinogenicity classification, the following approach is typically used:

- causal association in human epidemiological studies indicates a category 1 carcinogen classification
- causal association in animal studies (with no data or weak evidence from human studies) indicates a Category 2 carcinogen classification
- weak data from animal studies (*e.g.,* positive data by non relevant routes or in a poor study) and/or human studies, with some supporting evidence of mutagenicity indicates a Category 3 carcinogen classification

In reading across from one substance classified as a carcinogen to another, the relevance of the read across data has to be considered as well as the starting Category classification of the reference substance. A possible approach is shown below with examples from the read across for nickel substances.

VII. EXAMPLE: CARCINOGENICITY EFFECTS AND NICKEL SUBSTANCES: CLASSIFICATION AND RISK CHARACTERIZATION.

Nickel data availability for classification and risk characterization.
Data were available from several epidemiological studies of nickel refinery workers exposed to mixtures of nickel-containing substances and other confounders. Data were also available from three well-conducted cancer inhalation studies in rats with three different nickel compounds [1]. Based solely on the epidemiologic data, the EU Specialized Experts recommended nickel sulphate and chloride be classified as Category 1 carcinogens.

Read across for a Category 1 carcinogen
Presumably, the reference substance has a causal association in epidemiologic studies and no data or positive animal data in animal studies exist. The epidemiological studies should be strong enough to indicate that the reference substance being assessed[2] is responsible for excess cancer risks. For this substance, there may be quite a bit of information on the components of the mode of action, particularly if animal studies have been undertaken[3].

To read across from the reference substance to another substance under evaluation, a weight of evidence approach considering the various components of the mode of action could be undertaken.

Step 1.

- Evaluate data on any existing epidemiologic studies that included the substance under evaluation,
- Consider any animal evidence for carcinogenicity of the substance under evaluation,
- Assess presence of systemic versus local effects for substance under evaluation,
- Get data on factors that influence bioavailability at target cellular sites for cancer.
- If weight of evidence is strong of similar responses for reference and substance under evaluation

,

- *Consider read across with a category 1 carcinogen classification.*

[*For nickel substances, this could be exemplified by reading across from nickel sulphate and chloride to nickel nitrate (category 1 carcinogen), or for reading across from crystalline nickel subsulfide to nickel sulfide (category 1 carcinogen).*]

[1] *E.g.,* nickel sulphate hexahydrate, nickel monoxide calcined at high temperatures, and crystalline nickel subsulfide
[2] And not a mixture
[3] Note that this scenario did not exactly apply to nickel sulphate or nickel chloride since there were no epidemiological studies with single exposures to these substances and the animal cancer inhalation study with nickel sulfate was negative.

Step 2.

- Data on epidemiological studies does not exist for other substance or is very weak, but some animal data are available, or
- no human or animal data are available for substance under evaluation but data on mode of action is robust to indicate same effects as reference substance.

In this scenario, because there is a justifiable concern for the reference substance, but not too much supporting data for the other substance:

- *A read across to a lesser carcinogen classification (category 2 or 3) may be appropriate.*

[*For nickel substances, one example could be the read across from nickel monoxide to complex nickel-copper oxide. There are not enough data for the classification of nickel copper oxide as a category 1 carcinogen in its own but there are some animal injection studies that could provide supporting evidence for a category 2 carcinogen.*

Note. The EU TC C&L recommended a category 2 read across for many nickel containing substances in the absence of any supporting data, except water solubility.

Step 3.
No cancer/tumor data available for other substance, but some data on components of mode of action exist that suggest similarities (*e.g.,* data from bioaccessibility studies or even water solubility studies).

In this scenario:

- *Read across to a lesser carcinogen classification (category 3) may be appropriate*[4].

Read across from a Category 2 or 3 carcinogen.
In the case of substances classified as Category 2 and 3 carcinogens, the weight of evidence for carcinogenicity of the reference substance is weaker. The requirements for data needed to read across to the same carcinogenicity classification should be the same as mentioned above in steps 2 and 3. Otherwise, there is a risk of perpetuating the uncertainties, by directly reading across from a substance that has weak data to another with even less certain data.

Example, nickel metal has a category 3 carcinogen classification at the moment based on a couple of injection animal studies. To read across a Category 3 carcinogen classification from nickel metal to a NiTi alloy would be inappropriate unless animal supporting data would be available. Consistent with this idea, the EU TC CL & L did not read across from nickel metal to nickel alloys.

Test waiving
If for a given metal substance under evaluation, no data are available for steps 1-3. REACH may require the producer to undertake the minimal testing requirement (step 2-3) but the producer may potentially waive more thorough testing (step 1). In such a case the classification should be based on a realistic scenario using the minimal information available (step 2-3). While it is recognised that such cancer classification may be more conservative than when all test info would be available, it is

[4] a Category 3 may have been more appropriate read across based on existing data for hundreds of nickel substances, but instead the EU CL&L recommended a category 2 read across in the absence of any supporting data, except water solubility

not necessarily equal to a default worst case classification. Indeed the minimal testing requirement forms here the basis on which the decision based on the most plausible assumption should be made.

Example: For the case of nickel substances, read across from reference substances could result in category 2 or 3 along the criteria stated above. The default classification in the absence of read across data would be category 1 as applied to nickel carbonate hydroxide through a test derogation document. It should be made clear in this case that the classification given to nickel hydroxy carbonate is the result of test waiving derogation and therefore the weight of evidence implied in a Category 1 classification was not fulfilled in this case.

Read-across Example: Pb & Pb Compounds

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Acute Toxicity

Read across has been used under a select set of circumstances in the ongoing Voluntary Lead Risk Assessment. For purposes of testing derogation and acute toxicity classification, read across has been based upon evaluation of physical properties (e.g. particle size in dustiness testing) and water solubility. This strategic approach has been made possible by a set of circumstances that include:

- all compounds under evaluation were sparingly soluble in water
- acute oral toxicity data were available for a number of compounds under evaluation and indicated absence of toxicity up to the upper limit of ranges tested
- direct effects upon the skin or the lung (e.g. sensitization) were absent
- the particle size distribution of compounds was such that upper airway deposition would be predicted, to be followed by translocation to the gastrointestinal tract.- systemic effects following acute inhalation exposure would thus largely be dictated by the oral exposure route

Properties and acute oral toxicity lead and lead compounds

Substance	**CAS**	**Dustiness value [mg/g]**	**D50 [µm] phys. diameter(1)**	**D50 [µm] MMAD(2)**	**Solubility (mg/L)**	**Rat Oral LD50 (mg/kg/bw)**
lead metal powder	7439-92-1	189	12.7	33.7	230	-
lead oxide	1317-36-8	179	13.8	35.9	71	>2000
lead tetroxide	1314-41-6	8	4.5	14.0	88	>10,000
dibasic lead phthalate	69011-06-9	7	1.6	13.4	580	> 2000
basic lead sulphate	12036-76-9	37	1.7	15.4	19	-
tribasic lead sulphate	12202-17-4	13	1.8	12.9	102	> 5000
tetrabasic lead sulphate	12065-90-6	31	2.43	10.0	33	-
neutral lead stearate	1072-35-1	287	23.0	28.6	10	> 2000
dibasic lead stearate	12578-12-0	263	4.1	24.2	1.8	-
dibasic lead phosphite / sulphite	62229-08-7 12141-20-7	149	1.8	104.2	27	> 5000
polybasic lead fumarate	90268-59-0	134	1.4	55.4	71	-
basic lead carbonate	1319-46-6	33	2.1	6.0	0.2	> 2000
dibasic lead phosphite	12141-20-7	262	1.2	54.0	10	> 2000

Under this set of circumstances, read across was facilitated by a lack of acute effects for multiple compounds for either systemic toxicity resulting from ingestion routes of exposure or direct contact

toxicity. Water solubility was presumed to be an adequate, albeit potentially imperfect, indicator of compound dissolution on the skin (in sweat) or in the mucous of the pulmonary tract that suggested there was no reason to believe that compound specific differences existed that would modify this lack of toxicity.

Read across for acute oral toxicity was made possible by the absence of acute oral systemic effects – even though it was known that the oral bioavailability of the compounds being tested was in fact quite different and not reflected by differences in water solubility. The existing scientific literature has documented that the oral bioavailability of lead compounds varies by several orders of magnitude and as a function of the behavior of a given lead salt in the acidic condition of the gastrointestinal tract. The following table presents the conclusions regarding acute toxicity based upon read across following these principles. Measure values or conclusions are indicated in bold faced type. Conclusions based upon read across in accordance with the preceding principles are in *italics.*

Properties and acute oral toxicity lead and lead compounds

Substance	**Rat Oral LD50 (mg/kg/bw)**	**Inhalation LC50 (mg/L)**	**Dermal LD50 (mg/kg/bw)**	**Skin or lung sensitisation**
lead metal powder	*>2000*	*> 5*	*> 2000*	*None*
lead oxide	**>2000**	**> 5**	**> 2000**	**None**
lead tetroxide	**>10,000**	*> 5*	*> 2000*	*None*
dibasic lead phthalate	**> 2000**	*> 5*	**> 2000**	**None**
basic lead sulphate	>2000	*> 5*	*> 2000*	*None*
tribasic lead sulphate	**> 5000**	*> 5*	**> 10,000**	***None***
tetrabasic lead sulphate	*> 2000*	*> 5*	*> 2000*	*None*
neutral lead stearate	**> 2000**	*> 5*	*> 2000*	*None*
dibasic lead stearate	>2000	*> 5*	*> 2000*	*None*
dibasic lead phosphite / sulphite	**> 5000**	*> 5*	*> 2000*	*None*
polybasic lead fumarate	>2000	*> 5*	*> 2000*	*None*
basic lead carbonate	**> 2000**	*> 5*	*> 2000*	*None*
dibasic lead phosphite	**> 2000**	*> 5*	**>2000**	**None**

Repeated Dose Toxicity

Most toxicity studies of lead compounds in experimental animals have employed extremely soluble compounds such as lead acetate. Toxicity has further been observed in studies of exposed human populations, but the external dosimetry (administered dose) required to produce effects in humans is usually unknown. However, determination of lead levels in blood has proven to be a reliable index of systemic exposure and can serve as the basis for determining whether or not a level of exposure sufficient to produce systemic toxicity has been attained.

Prediction of the toxic potential of different lead compounds based solely upon physical or chemical properties of individual lead compounds has proven difficult due to discrepancies between parameters such as water solubility and the relative bioavailability of different lead compounds following the more common routes of exposure (e.g. ingestion). The following table presents the aqueous solubility of a number of lead compounds and contrasts their typical relative bioavailability to that of lead acetate, one of the most soluble and bioavailable compounds known. While extremely water insoluble lead compounds can have low bioavailability, some can exhibit bioavailability that approaches that exhibited by lead acetate. The failure of the water solubility of compounds to correlate with relative bioavailability is due to the complex acidic conditions within the stomach and variability of bioavailability of as a function of decreasing particle size. As particle size decreases, the resistance of otherwise stable lead compounds in the gastrointestinal tract will be diminished. Relatively oral bioavailability will further me modulated by "matrix" effects that can be exerted by lead-containing foods or soils that may be ingested.

Comparison of water solubility and bioavailability (oral) relative to that of lead acetate (in %)

Compound	**Water Solubility (mg/L)**	**% Bioavailability (relative to lead acetate)**
Lead Acetate	400,000	100
Lead Carbonate	0.2	100
Lead Oxide	71	60
Lead Sulphide	120	<1
Lead Metal Powder	230	<10

The relative bioavailability of different lead compounds can be determined by animal feeding studies. *In vitro* test systems have also been validated that can predict the bioavailability of lead contained in matrices such as soil but cannot yet be applied to predict the bioavailability of pure compounds.

The costs of conducting animal feeding studies, and the multitude of modifying factors that can alter bioavailability, are such that estimates of the presence or absence of risk of repeated dose toxicity is generally assessed from studies of exposed human populations and the level of lead that is observed in blood. If the level of lead in blood is above that associated with systemic toxicity, then risk of repeated dose toxicity is presumed. If it is lower, the lack of risk is assumed.

CMR Endpoints

Evaluation of CMR endpoints, and read across issues associated with them, has proven to be challenging within the context of the existing voluntary lead risk assessment.

Mutagenicity/Genotoxicity

Highly soluble lead compounds have been shown to be mutagenic and genotoxic *in vitro*, but responses observed are generally weak and appear to be mediated by indirect mechanisms (e.g. inhibition of DNA repair, alteration of oxygen radical metabolism), presumably through the action of the lead cation. Concentrations required to elicit these effects are usually high (mM concentration range) and beyond the solubility of most lead compounds being evaluated to for risk assessment purposes. On the basis that mechanism(s) of mutagenic action are presumed to be mediated by the lead cation, it has yet to be determined if insoluble compounds can realistically be expected to exhibit activity *in vitro*. Testing *in vivo* has similarly been conducted almost exclusively with highly water soluble compounds, yielding weak and inconsistent results that have been both difficult to interpret and to extrapolate to insoluble compounds.

Carcinogenicity

Soluble lead compounds produce kidney cancer in rodents when administered at high doses and, as with mutagenicity, appears to be mediated by indirect mechanisms. Testing of insoluble compounds (oral gavage studies with lead metal powder, inhalation studies with lead oxide) have produced negative results. It thus remains to be determined how high dose effects obtained in animals through the administration of soluble/bioavailable compounds can be extrapolated to insoluble or nonbioavailable compounds. Extrapolation between compounds based upon the blood lead levels that can be produced is being attempted, but is complicated by a lack of blood lead measurements in most cancer bioassays.

Reproductive Toxicity

Lead compounds are known human and animal reproductive toxicants, most notably inducing developmental deficits at relatively low levels of exposure. While it is conceivable that some lead compounds may have bioavailability that is sufficiently limited so as to restrict their reproductive toxicity, the doses required to produce developmental effects are sufficiently low to support a presumption that most, if not all, lead compounds should be classified as known human developmental toxicants.

Grouping of Petroleum Substances

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Grouping of Petroleum Substances

The purpose of this paper is to describe the historical approach for the grouping of petroleum substances which has been applied in the context of the Existing Substances Regulation 793/93 and for classification under the Dangerous Substances Directive 67/548.

Background

There was a need to arrange approximately 660 petroleum substances listed on EINECS, into smaller groups to assist in the collection, summary and evaluation of hazard data for the purpose of satisfying the requirements of the Existing Substances Regulation (EU Council regulation 793/93 of 23 March 1993, published in the Official Journal L84 on 5 April 1993). The grouping approach developed for that purpose was also used by the EC C&L working group in their assessment for classification for carcinogenicity of petroleum substances (21st adaptation to technical progress of the Dangerous Substances Directive, published in the Official Journal L 381 on 31.12.1994). In addition, the oil industry association CONCAWE has employed this grouping approach in the voluntary risk assessment of gasoline, which has been presented to and discussed by TCNES at its meeting in December 2004, and in the drafting of industry guidance on the classification and labelling of petroleum substances.

Petroleum substances are of unknown and variable composition and are regarded as typical UVCBs (substances of Unknown or Variable compositions, Complex reaction products or Biological materials). They can therefore only be described in general terms and hence the assigned CAS numbers do not describe them precisely in terms of their exact chemical composition. However, the CAS definitions for petroleum substances identify the starting material, the last processing step that the substance underwent during its manufacture and, in many cases, an indication of key physico-chemical properties such as boiling range, and carbon number range.

For these reasons a different approach was required for grouping the petroleum substances compared to the classical grouping approach used for well defined chemical substances.

Rationale and grouping of petroleum substances

Since it was not possible to characterise the petroleum substances in terms of their exact chemical composition it was decided to group them according to the process by which they are being manufactured. It was reasoned that similar starting materials that underwent similar modification and/or separation processes would result in streams of broadly similar composition.

The resulting groups and sub-groups of petroleum substances were published in the Official Journal L84 on 5 April 1993. Descriptions for each of the groups or sub-groups are described in Table 1.

Use of the grouping methodology

The creation of groups (and sub-groups) of petroleum substances allows the determination/prediction of their intrinsic hazard properties through application of a variety of techniques, such as use of test data, read-across, QSAR modelling, and marker substances (i.e., 1,3-butadiene, benzene, polycyclic aromatics). It should be noted that when read-across is applied to a group/sub-group, all substances included in the groups/sub-groups are presumed to exhibit similar intrinsic hazard properties based on similarities in their physico-chemical properties. This grouping methodology was also developed with the desire to avoid further animal testing and was confirmed by the Commission in the Official Journal L84 on 5 April 1993.

As illustrated by the following example, a combination of the use of hazard data, read-across and markers was applied for the classification of petroleum substances for carcinogenicity. It should be

noted that markers have been developed to facilitate the classification for carcinogenicity; classifications for other endpoints are largely based on read-across from data on related petroleum substances.

Example of the approach used for the classification for carcinogenicity

For some of the individual substances in the petroleum substance groups/sub-groups listed in Table 1, results of carcinogenicity studies were available. However, it was also recognized that some refining processes to which the distillation fractions of crude oil were subjected could result in the occurrence of varying levels of individual constituents, which themselves were known to be carcinogenic. Therefore, it was decided to use a combination of available carcinogenicity data and knowledge of the occurrence of known carcinogenic substances in the streams during the assessment of classification for carcinogenicity.

The carcinogens that were known to occur in petroleum substances and the concentrations used as indicators for classification were 1,3-butadiene (≥0.1%), benzene (≥0.1%) and IP-346 DMSO extractables (≥3%); the latter is a surrogate for polycyclic aromatic hydrocarbons (PAHs), which was correlated with results from experimental studies. Since the groups of petroleum substances originate from different processes, this determined which carcinogenic constituents were likely to be present in substances from the respective groups.

The following information was used by the EU C&L working group to assess the carcinogenicity classification of each of the following groups of petroleum substances. The classifications represent the *harmonised EU Classifications which appear in Annex 1 to the Dangerous Substances Directive (67/548 EC)*. They are examples illustrating how the techniques were applied:

Crude Oil (group 1) contains a number of carcinogens, notably benzene and 4 to 6 fused ring polycyclic aromatic hydrocarbons (PAHs).
Classified as a Category 2 carcinogen on the basis of available animal studies.

Petroleum Gases (group 2) derive from crude oil distillation and cracking processes. The latter can give rise to the presence of 1, 3-butadiene. For those gases which are condensed to give LPG, either butadiene extraction or hydrofinishing is normally practiced reducing the concentration of butadiene to negligible levels.
All Group 2 substances are classified as Category 1 carcinogens unless the concentration of 1,3-butadiene is <0.1%.

Gasoline Streams (groups 3A to 3G) consist of petroleum substances composed of aliphatic and aromatic hydrocarbons including benzene.
All Group 3A to 3G substances are classified as Category 2 carcinogens unless the concentration of benzene is <0.1%.

Kerosine Streams (groups 3H to 3J) all contain promoters. Do not contain significant amounts of benzene or PAHs
On the basis of animal studies, Group 3H to 3J substances are not classified for carcinogenicity.

Gas Oil Streams (groups 4 and 5) all contain promoters. Straight run gas oils (group 4A) do not contain significant concentrations of PAHs. Cracked gas oils (group 4B) contain PAHs. Other gas oils (group 5B), with the exception of distillate fuels – see below, are assumed to be carcinogenic unless information is available to contrary on the refining history.

On the basis of animal studies, Group 4A substances are not classified for carcinogenicity.
On the basis of animal studies, Group 4B and 5B substances are classified as Category 2 carcinogens.
Based on available information the EU C&L working group classified four distillate fuel oils (fuels diesel, fuel oil number 2, and fuel oil number 4 and fuels diesel number 2) as Category 3 carcinogens.

Heavy Fuel Oil Streams (group 6A) consist of heavy distillates or residual fractions from distillation or cracking processes and contain saturated, aromatic and olefinic hydrocarbons. Some of these substances contain significant concentrations of PAHs.
All Group 6A substances are classified as Category 2 carcinogens on the basis of animal studies.

Lubricating Greases (group 6B) consist of lubricant base oils and thickeners. The PAH content of greases is dependent on the nature and severity of the treatment processes to which the base oils have been subjected.
Classification of Group 6B substances is dependent on the classification of the base oil.

Lubricant Base Oils (groups 7A to 7C) consist of vacuum distillates and oils obtained from vacuum residues. Their PAH content is dependent on the nature and severity of the treatment process to which they have been subjected.
On the basis of human data, all Group 7A substances are classified as Category 1 carcinogens.
On the basis of animal studies, all Group 7B substances are not classified for carcinogenicity.
All Group 7C substances are classified as Category 2 carcinogens unless the IP-346 DMSO extractables (surrogate for PAH levels) are < 3%.

Lessons Learned

The classical OECD guidance for the formation of chemical categories is appropriate for the application on chemicals with well-defined chemical structure. As discussed it is not considered suitable however for addressing categories of UVCB substances, which by their very definition are of unknown or varying composition. The grouping approach developed by CONCAWE, based on similarities in refinery manufacturing process and physico-chemical properties, provides a pragmatic solution, which has been applied to the evaluation of toxicological, ecotoxicological and physico-chemical hazards of petroleum substances, whilst minimizing unnecessary animal testing.

Table 1
Description of groups of petroleum substances

Group (and sub-group) number*	Name of the group (or subgroup)		description
1	CRUDE OIL	Crude oil	Raw petroleum oil obtained in its natural state from the ground (excluding hydrocarbons from shale and coal) and containing aliphatic, alicyclic, and aromatic hydrocarbons, with small quantities of nitrogen, oxygen and sulphur compounds.
2	PETROLEUM GASES	Petroleum gas	Streams obtained from crude oil distillation, cracking processes and tail gases, containing saturated and/or olefinic hydrocarbons mainly in the range C_2 to C_5
3A	LOW BOILING POINT NAPHTHAS (GASOLINES)	Low boiling point naphtha	Streams obtained from the atmospheric distillation of crude oil and containing saturated and aromatic hydrocarbons, mainly in the range C_4 to C_{12} and boiling in the range ca. -20 to 230°C.
3B	LOW BOILING POINT NAPHTHAS (GASOLINES)	Low boiling point modified naphtha	Streams obtained by alkylation (catalytic reaction), isomerisation (catalytic conversion) and solvent extraction, and containing saturated hydrocarbons, mainly in the range C_5 to C_{12} and boiling in the range ca. 35 to 230°C.
3C	LOW BOILING POINT NAPHTHAS (GASOLINES)	Low boiling point cat-cracked naphtha	Streams obtained from the catalytic cracking of heavy distillates into lighter fractions, and containing saturated, olefinic and aromatic hydrocarbons, mainly in the range C_4 to C_{12} and boiling in the range ca. -20 to 230°C.
3D	LOW BOILING POINT NAPHTHAS (GASOLINES)	Low boiling point cat-reformed naphtha	Streams obtained from the catalytic reforming of mainly n-alkane and cycloparaffinic feedstocks into aromatic and branched chain hydrocarbons, mainly in the range C_5 to C_{12} and boiling in the range ca. 35 to 230°C.
3E	LOW BOILING POINT NAPHTHAS (GASOLINES)	Low boiling point thermally cracked naphtha	Streams obtained by the high temperature splitting of heavy distillates into lighter fractions, and containing saturated, olefinic and aromatic hydrocarbons, mainly in the range C_4 to C_{12} and boiling in the range ca. -20 to 230°C.
3F	LOW BOILING POINT NAPHTHAS (GASOLINES)	Low boiling point hydrogen treated naphtha	Streams obtained by the catalytic reaction of feedstocks with hydrogen to remove unsaturated and organic sulphur compounds, and containing mainly saturated hydrocarbons, mainly in the range C_4 to C_{12} and boiling in the range ca. -20 to 230°C
3G	LOW BOILING POINT NAPHTHAS (GASOLINES)	Low boiling point naphtha – unspecified	Streams obtained by processes such as steam and hydro cracking and sweetening, and containing saturated, aromatic and olefinic hydrocarbons, mainly in the range C_4 to C_{12} and boiling in the range ca -20 to 230°C.
3H	STRAIGHT RUN KEROSINES	Straight run kerosine	Streams obtained from the atmospheric distillation of crude oil, and containing saturated and aromatic hydrocarbons, mainly in the range C_9 to C_{16} and boiling in the ca. range 145 to 300°C.
3I	CRACKED KEROSINES	Cracked kerosine	Streams obtained from processes involving the cracking of hydrocarbon feedstocks, and containing saturated, olefinic and aromatic hydrocarbons, mainly in the range C_8 to C_{16} and boiling in the ca. range 90 to 290°C.
3J	OTHER KEROSINES	Kerosine – unspecified	Streams obtained from processes not sufficiently defined to enable them to be placed in sub-groups 3I or 3J and containing saturated, aromatic and olefinic hydrocarbons, mainly in the range C_7 to C_{16} and boiling in the range ca. 90 to 290°C.
4A	STRAIGHT RUN GAS OILS	Straight run gas oil	Streams obtained from the atmospheric distillation of crude oil, and containing saturated and aromatic hydrocarbons, mainly in the range C_9 to C_{25} and boiling in the range ca. 150 to 400°C.
4B	CRACKED GAS OILS	Cracked gas oil	Streams obtained from processes involving the cracking of hydrocarbon feedstocks, and containing saturated, olefinic and aromatic hydrocarbons, mainly in the range C_9 to C_{25} and boiling in the range ca. 150 to 400°C.
5A	VACUUM GAS OILS	Vacuum gas oil	Streams obtained from the vacuum distillation of atmospheric residues, and containing saturated and aromatic hydrocarbons, mainly in the range C_{11} to C_{25} and boiling in the range ca. 200 to 450°C.
5B	OTHER GAS OILS	Gas oil – unspecified	Streams obtained from processes not sufficiently defined to enable them to be placed in groups 4A, 4B or 5A and containing saturated, aromatic and olefinic hydrocarbons mainly in the range C_9 to C_{25} and boiling in the range ca.

Group (and sub-group) number*	Name of the group (or subgroup)		description
			150 to 450°C.
6A	HEAVY FUEL OIL COMPONENTS	Heavy fuel oil	Streams obtained as either distillates or residues from distillation and cracking processes, and containing saturated, aromatic and olefinic hydrocarbons mainly in the range C_9 to C_{50} and boiling in the range ca. 160 to 600°C.
6B	LUBRICATING GREASES	Grease	A complex combination of hydrocarbons mainly in the range C_{12} to C_{50} and containing organic compounds of alkali metals, alkaline earth metals and/or aluminium.
7A	UNREFINED/ACID TREATED OILS	Unrefined or mildly refined base oil	Untreated and acid treated streams obtained from the vacuum distillation of atmospheric residues, and containing saturated and aromatic hydrocarbons, mainly in the range C_{15} to C_{50}.
7B	HIGHLY REFINED BASE OILS	Highly refined base oil	Streams obtained by (a) severe refining of vacuum distillates to remove aromatic hydrocarbons or (b) the treatment of vacuum residues, and containing saturated and aromatic hydrocarbons, mainly in the range C_{12} to C_{50}.
7C	OTHER LUBRICANT BASE OILS	Base oil – unspecified	Streams obtained from vacuum distillates, vacuum residues and atmospheric distillation residues by processes such as solvent extraction or hydrogenation, and containing saturated and aromatic hydrocarbons, mainly in the range C_{10} to C_{50}.
8	RESIDUAL AROMATIC EXTRACTS	Residual aromatic extract	Streams obtained from the solvent extraction of vacuum residues, and containing saturated and aromatic hydrocarbons, mainly in the range > C_{25}.
9A	UNTREATED DISTILLATE AROMATIC EXTRACTS	Distillate aromatic extract	Streams obtained from the solvent extraction of vacuum distillates, and containing mainly aromatic hydrocarbons, mainly in the range C_{15} to C_{50}.
9B	TREATED DISTILLATE AROMATIC EXTRACTS	Distillate aromatic extract (treated)	Streams obtained by subjecting untreated aromatic extracts from vacuum distillates to processes such as hydrogenation, and containing predominantly saturated and aromatic hydrocarbons, mainly in the range C_{15} to C_{50}.
10	OTHER AROMATIC EXTRACTS	Aromatic extract – unspecified	Stream obtained by the solvent extraction of straight run gas oils, vacuum gas oils and distillation residues etc., and containing saturated and aromatic hydrocarbons, mainly in the range C_9 to C_{30} and boiling in the range ca. 150 to 450°C.
11A	PARAFFIN AND HYDROCARBON WAXES	Petroleum wax	Streams obtained as the insoluble phase from the solvent treatment of atmospheric and vacuum distillates or vacuum residues, and containing saturated straight and branched chain hydrocarbons, mainly in the range C_{20} to C_{50}.
11B	FOOTS OILS	Foots oil	Streams obtained as the liquid phase in the separation of paraffin wax from slack wax, and containing mainly branched chain saturated hydrocarbons, mainly in the range C_{20} to C_{50}.
11C	SLACK WAXES	Slack wax	Streams obtained by the solvent dewaxing of vacuum distillates, and containing straight and branched chain saturated hydrocarbons, mainly in the range >C_{20}.
11D	PETROLATUMS	Petrolatum	Streams obtained by the solvent dewaxing of vacuum residues, and containing mainly branched chain saturated hydrocarbons, mainly in the range >C_{20}.
12	USED AND RE-REFINED OILS	Used or re-refined oil	Spent formulated oils derived from various uses, most of which are treated by processes such as clay percolation, hydrogenation and distillation, and mainly in the range C_{15} to C_{50}.
13	BITUMEN	Bitumen or vacuum residue	Streams obtained as residues from vacuum distillation and cracking processes, some of which are subjected to further processing, and containing saturated and aromatic hydrocarbons mainly in the range >C_{25}
14	PETROLEUM COKE	Petroleum coke	Granular or needle like substances, basically carbon, contained by the high temperature decomposition of heavy oils. May contain some high molecular weight hydrocarbons.

*According to the Official Journal L84 of 5 April 1993

Case Study on Polyols

Mike Jeffs

CEFIC

September 2006

ISOPA INPUT RIP3.3-2 TASK 3

CASE STUDY: POLYOLS

Introduction

ISOPA has an interest in a range of polyols that have been defined by the European Union as 'no longer polymers'. The polyols are composed of an initiator, together with a chain or chains of propylene oxide (PO) or mixtures of ethylene oxide (EO) and PO with a range of molecular weights. They contain a common functional group - the hydroxyl group of which large numbers are present in each chemical entity. Because of their polymeric nature and apparent lack of hazard these substances have hitherto, not been extensively tested for toxicity and ecotoxicity. ISOPA is now addressing this problem in anticipation of the implementation of the EU REACH (Registration, Evaluation and Authorisation of CHemicals) legislation [1] by developing a case for read across in respect of health effects for members of a set of polyols.

The read across case for the ISOPA polyols essentially follows steps 1-5 of the process proposed by the OECD [2].

Step 1: Identification of the compounds of interest

ISOPA has grouped this product range into 10 categories, based on 9 initiators. The first nine categories are propoxylated derivatives of sucrose, propylidine trimethanol, D-glucitol, pentaerythritol, nitrilotriethanol, ethylenediamine, *o*-toluenediamine, glycerol and propylene glycol. The tenth category is a mixed ethoxylated/propoxylated derivative of ethylenediamine. Their CAS and EINECS numbers are shown in Table 1.

Step 2: Collection of data for each category member

Available test reports on individual polyols were collected from various ISOPA members. Literature searches were conducted and data collected concerning the toxicity of each initiator and of the repeating units (poly-PO and poly-EO). The data collection included undertaking a limited number of basic studies o fill obvious gaps.

Steps 3 and 4: Evaluation of available data for adequacy and construction of a matrix of data availability

Only studies of at least a minimal acceptable quality for classification and labelling (i.e. Klimisch categories 1 and 2, as determined by ISOPA) were included. The information for each of the ten categories was set out in individual Microsoft Excel spreadsheets in a single file. The individual end points were given on the x-axis and the average molecular weight on the y-axis, beginning with information on the initiator as the first line and with each successive line being for the next higher molecular weight average. In general, no entry means that no study was available and the entry

gives the key toxicity information. In this sense the approach used was more detailed than that set out at step 4 of the OECD proposed process.
The polyol chain units (propoxy and ethoxy) were also examined; they were found to be non-toxic.

In a second approach, the relative bioavailabilities of each component within each of the 10 groups were calculated using QSAR techniques [3, 4]. In all cases, the calculated bioavailability of the individual molecular species decreased with increasing molecular weight.

Step 5: Assessment of the validity of the category

The polyol chain units were found not to be intrinsically toxic. It was therefore felt to be reasonable to consider the polyol as a common functional group (group 1.4.1 of Annex IX), and to consider the 10 polyols simply on the basis of their different initiators. A review of the toxicology of the 9 initiators showed that 7 of them were essentially non-toxic, whilst two - ethylenediamine and *o*-toluenediamine - exhibited some distinct and separate toxic properties, principally based on their free amino functions. These latter two initiators have been termed "reactive" initiators and the three polyols derived from them have been placed in two separate sub-categories reflecting the different toxic properties of their initiators. In both cases the toxicity rapidly decreased with increasing molecular weight of the polyol and they represent 'a constant pattern in the changing of the potency of the properties across the category'.

The toxicity data from polyols derived from the 7 "non-reactive" initiators was set against their calculated bioavailability profiles. Generally, the bioavailablility decreased with increasing molecular weight, thus, if the strength of any toxic effect depended on bioavailability it would be manifest at the lowest molecular weight and would decrease rapidly with increasing molecular weight. This would be in line with the assumption, normally made, that, for the majority of foreign compounds absorption depends on passive diffusion, although it is accepted that other mechanisms (e.g. facilitated diffusion, active transport) may be relevant when considering substances mimicking dietary constituents. The polyols derived from glycerol and propylene glycol were clear exceptions. They showed a systemic toxicity to rats over certain MW ranges. It was concluded that they are probably absorbed by a mechanism based on the absorption of dietary fats and fatty acids [5, 6]. It is a qualitative structure-activity relationship based on knowledge of mechanisms of absorption. The polyols from glycerol and propylene glycol were therefore placed in a separate sub-category from the other 5 "non-reactive" polyols.

The four sub-categories can be summarised as:

- Propoxylated derivatives from five essentially non-toxic initiators (sucrose, propylidine trimethanol, D-glucitol, pentaerythritol and nitrilotriethanol). The polyols derived from these initiators are also essentially non-toxic
- Propoxylated and propoxylated/ethoxylated derivatives obtained from the 'reactive' initiator ethylenediamine, *o*-toluenediamine. These polyols showed decreased toxicity with increasing molecular weight as the effect of the initiator toxicity was 'diluted out'.
- Propoxylated derivatives obtained from the 'reactive' initiator *o*-toluene diamine. These polyols showed decreased toxicity with increasing molecular weight as the effect of the initiator toxicity was 'diluted out'.
- Propoxylated substances derived from the initiators glycerol and propylene glycol over certain MW range. These polyols are probably absorbed by a mechanism other than passive diffusion.

Step 6: Preparation of a test plan for the four sub-categories

Following our review of the toxicology of the polyols within the four sub-categories defined above, we are now in a position to identify appropriate test substances to deliver the maximum level of useful information suitable for classification and labelling, with minimum cost and use of experimental animals. For some toxicological endpoints, it may be possible to combine some of the sub-categories for the purposes of read-across, thereby further reducing cost and use of animals.

Conclusion

We have demonstrated that, in the case of the polyols, the grouping principle based on (a) essentially non-toxic initiators and (b) common functional groups, meets the OECD categorisation process criteria and can be used in the "read across" for Annex IX of the proposed REACH regulation.

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Table 1. CAS and EINECS numbers of initiators and their respective polyols

Initiator	CAS	EINECS	Polyol chain	CAS No of Polyol	NLP
Ethylenediamine	107-15-3	203-468-6	propoxylated	25214-63-5	500-035-6
			ethoxylated and propoxylated	26316-40-5	500-047-1
D-Glucitol	50-70-4	200-061-5	propoxylated	52625-13-5	500-118-7
Glycerol	56-81-5	200-289-5	propoxylated	25791-96-2	500-044-5
2,2',2''-Nitrilotriethanol	102-71-6	203-449-8	propoxylated	37208-53-0	500-094-8
Pentaerythritol	115-77-5	204-104-9	propoxylated	9051-49-4(25214-63-5)*	500-030-9
Propane-1,2-diol	57-55-6	200-338-0	propoxylated	25322-69-4	500-039-8
Propylidynetrimethanol	77-99-6	201-074-9	propoxylated	25723-16-4	500-041-9
Sucrose	57-50-1	200-354-9	propoxylated	103513-09-3	500-029-3
Toluene-2,3-diamine	2687-25-4	220-248-5	propoxylated	63641-63-4	500-158-5

* two CAS numbers given for this substance

Approaches to Chemical Categorization: An Illustrative Example of Approaches Used by the Fragrance Industry

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Approaches to Chemical Categorization: An Illustrative Example of Approaches Used by the Fragrance Industry

Scope

This document is illustrative of the approaches used by the fragrance industry to categorize chemicals for evaluation of human health and environmental endpoints. Specifically, it considers an approach proposed by the fragrance industry for evaluating the systemic, reproductive, developmental and genotoxic effects and environmental toxicity and fate of a group of 22 monoterpene primary alcohols and aldehydes and structurally related esters and acetals. Other effects arising from topical exposure such as sensitisation or irritation or other routes (for example via inhalation) are not considered, however similar criteria and considerations for chemical categorization may also be applied to these and other toxicological endpoints.

Introduction

The safety evaluation of fragrance ingredients presents unique challenges principally due to the fact that there are over 2,600 chemically defined fragrance ingredients and naturally occurring mixtures (e.g., essential oils) in commerce, the vast majority of which are used at low levels. These substances are used to produce a wide range of fragrances intended for use in cosmetics, fine fragrances, household, personal care and other perfumed products. A majority of these substances are used in food flavours and are present naturally in a traditional diet.

A large number of fragrance substances are found in plants as products of biosynthetic pathways such as the isoprene and shikimic acid pathways and contain the same carbon skeleton backbone and functionality, i.e. they are close structural relatives. Both aquatic and terrestrial animals have evolved an array of biochemical processes to safely dispose of these naturally occurring substances and structurally related analogs

Given the large number and commonality of fragrance substances, chemical grouping can effectively be used to assess their hazards and risks.

For more than 40 years, the fragrance industry, through its human health and environmental safety research institute (Research Institute for Fragrance Materials or RIFM) and its various trade associations, has used chemical grouping and read across methods to objectively assess the safety of chemicals used as fragrance ingredients in consumer products. These approaches have included grouping of chemicals in order to assess their safety for consumer use in collaboration with the RIFM Expert Panel and compliance with various international regulatory initiatives (e.g., US High Production Volume program and, in

preparation for REACH, the Strategic Partnership on REACH Testing). Flavours have also been assessed using similar methodology to provide safety assessments for JECFA in support of, among other initiatives, EU activities under FLAVIS and EFSA.

Despite encompassing more than 2,600 discrete chemicals, the majority of fragrance materials can be classified into a relatively few basic structural groups which can be further subdivided based on structural moiety most likely to be significant toxicologically and rendering the groups as similar as possible between molecules by structural type (Bickers et al., 2003a). It is key to note that fragrance chemical groups contain structurally related substances that participate in common metabolic pathways and exhibit similar toxicologic potential. As an example, RIFM in consultation with the RIFM Expert Panel, has recently published an assessment for the chemical group containing linalool and linalyl esters (Bickers et al., 2003b) and three key cinnamyl fragrance ingredients (Bickers et al., 2005). Another example is the US EPA High Production Volume Program, where essentially all fragrance chemicals encompassed by the Program were organized by the fragrance industry into groups, which were accepted for evaluation by the EPA.

Criteria for Chemical Group Approach

A limited number of chemical groups exist for flavor/fragrance substances because approximately 90% of the chemicals are simple, low molecular weight substances derived from well-recognized plant biosynthetic pathways consisting of only carbon, hydrogen and oxygen.

In order to apply the chemical group approach for fragrance ingredients, certain criteria can be applied to identify members of a chemical group.

1) Members of the group should contain the same or homologous carbon skeletal backbone structure.

2) Members of the group should contain common functional groups or functional groups that participate in common biochemical pathways yielding metabolites that are of lower toxic potential than the members of the chemical group. For example, a group may contain esters, acetals, alcohols, aldehydes and carboxylic acids provided that data are available to show that the esters and acetals rapidly hydrolyze *in vivo* to yield their corresponding alcohols and aldehydes that subsequently the alcohols and aldehydes are efficiently oxidized to the less toxic carboxylic acid derivatives. Systemic exposure levels should typically be well below those required to saturate available detoxication pathways and thresholds of toxicological concern.

3) Toxicity data among representative members of the group should show consistent effects and similar toxic potential for the endpoint considered. For example, it is justifiable to consider terpene hydrocarbons in a chemical group, since studies on representative members of the group (limonene, myrcene, alpha-pinene, and camphene) show a common target organ (kidney) and toxic levels within the same order of magnitude following systemic exposure.

Structural homologies allow hazard and risk issues to be considered for several materials within the context of the information that exists for the representative members of the chemical group. In many cases existing information for a structural group may obviate the need to submit a particular individual substance to full toxicological testing and QSAR and expert judgment can be applied to reduce uncertainty. In other cases, it may be necessary to test one or more particular members of a structural class to obtain a more robust assessment of the class as a whole.

The application of these principles and criteria for the identification and organization of a chemical group of fragrance materials is illustrated for a group of 22 monoterpene primary alcohols and aldehydes and structurally related esters and acetals. The chemical group also includes one natural-occurring complex mixture (lemongrass oil) whose main constituents are members of this chemical group and account for the majority (>80%) of the mass of the natural but other natural substances may also be relevant. The chemical grouping exercise is intended to demonstrate the type and extent of data required to support the formation of a chemical group, and to provide the basis for assessing the hazards and risks associated with use of members of the chemical group. An accompanying data grid provides an overview of the available data for the monoterpene primary alcohol/aldehyde/ester/acetal group (Attachment 1).

Chemical Group Approach

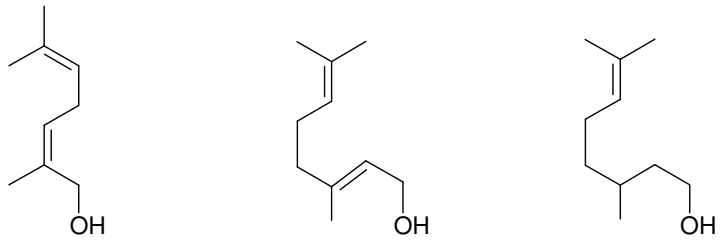
Monoterpene Primary Alcohols and Aldehydes and Related Esters and Acetals

Introduction

Chemical substances

The twenty-two monoterpene alcohols, aldehydes esters and acetals are all derived from the same branched-chain monoterpene C_{10} carbon skeleton (3,7-dimethyl-1-octyl) containing an oxygenated function group on C_1. The alcohols in this group are commonly recognized as geraniol, nerol and

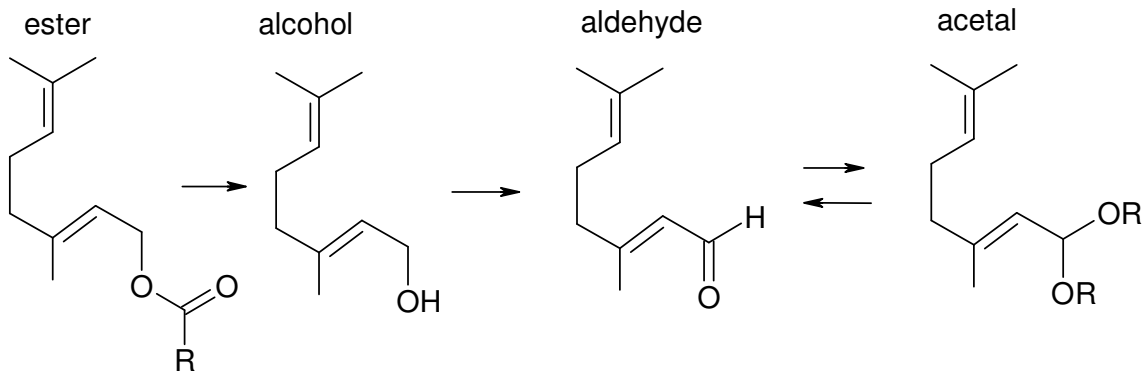
citronellol. Structurally, they are either *cis-trans* isomers (nerol/geraniol) or a dihydro derivative (citronellol).



nerol geraniol citronellol

Although they contain different oxygenated functional groups at the terminal carbon, rapid hydrolysis in animals converts the esters and acetals into the corresponding alcohols and aldehydes that then participate in the common metabolic pathways of detoxication (see Figure 1). Differences in structure among members of the group include: 1) the length of the aliphatic chain in the carboxylic acid component of the ester or alcohol component of the acetal, 2) the length and position of one of the alkyl substituents in the monoterpene, and 3) the presence or absence of unsaturation within the monoterpene carbon skeleton. Although the latter difference may seem important if the unsaturation involves an alpha,beta-unsaturated aldehyde, metabolic data exist to show that these substances do not efficiently conjugate with glutathione and, therefore, are not associated with the systemic toxic effects due to cellular glutathione depletion and oxidative stress (Diliberto *et al.*, 1990; Parke and Rahman, 1969; Boyer and Petersen, 1990; Chadha and Madyastha, 1982; NTP, 991)

Figure 1



Naturally-occurring botanical mixture of variable composition

In addition to chemically-defined substances, essential oils (botanical UVCB's) derived from plants are important fragrance materials. Although the composition is variable, the variability is limited by the fact that key constituents must be present at sufficient concentrations to exert the required fragrance effect. Hence, citronella oil and lemongrass oil are effective as fragrances only when they contain a complex mixture containing mainly monoterpene primary alcohols, aldehydes and related esters. Greater than 80% of commercial West or East Indian types of lemongrass oil is composed of a mixture of monoterpene branched-chain primary alcohols (geraniol, nerol, citronellol), aldehydes (citral as a mixture of geranial and neral), acids, and related esters (geranyl acetate). The majority of the remaining substances are terpene hydrocarbons (e.g., limonene and myrcene) that exhibit a toxic potential similar to that of the members of this chemical group. Therefore, it is not unreasonable to conclude that metabolism and toxicity data representative of the chemical group is also representative of a mixture of the chemical group, i.e., the essential oil.

Categorization Criteria

In order to qualify for membership in the chemical group, members must meet the aforementioned criteria. Substances must not only show similar biochemical fate, but the toxic potential for representative members of the group (esters, alcohols, acetals and aldehydes) should be consistent thereby defining the applicability domain and boundary substances for the different endpoints required.

The discussion below attempts to evaluate the consistency and strength of the data within the category in this context.

Hydrolysis of monoterpene esters and acetals and metabolism of monoterpene alcohols and aldehydes

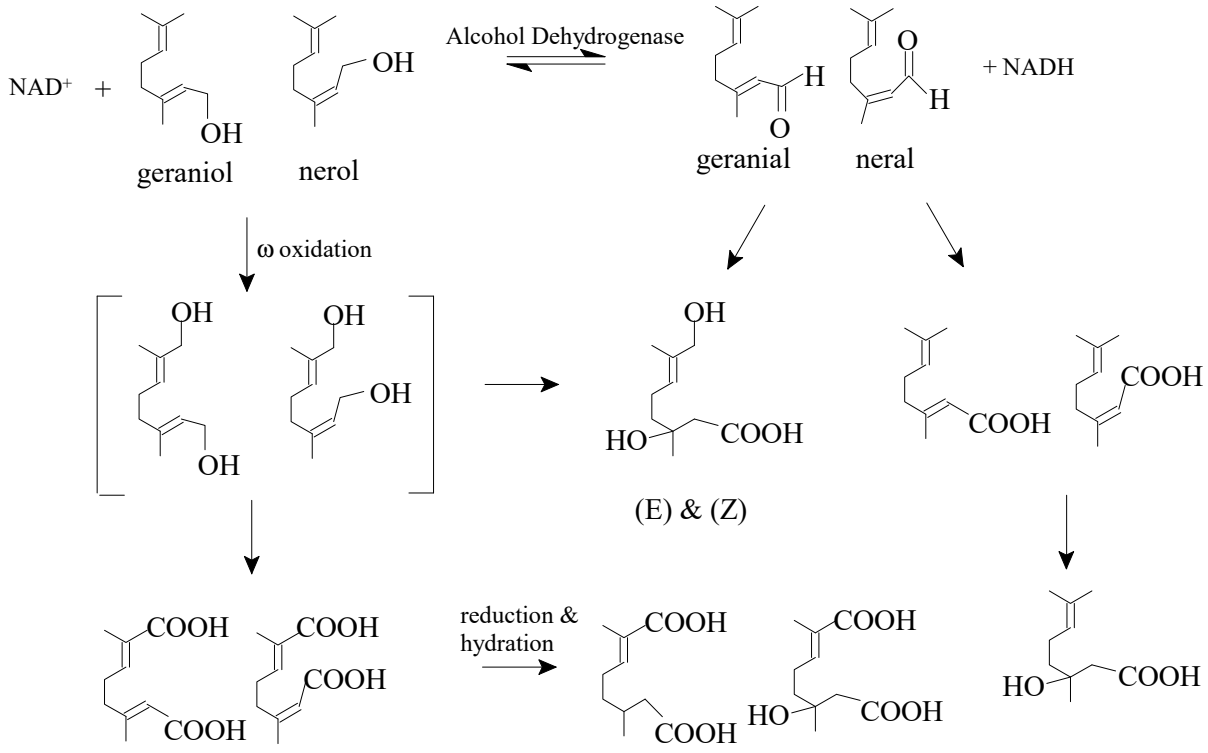
Monoterpene esters have been demonstrated to hydrolyse to the corresponding terpene alcohol in both terrestrial and aquatic species such as fish and subsequently the alcohols are metabolized to the corresponding aldehydes, which are then converted into polar excretable products (Diliberto *et al.*, 1990; Ishida *et al.*, 1989). Acetals are also hydrolysed to aldehydes that enter the same pathway (Morgareidge, 1962; Vicchio *et al.,* 1989).

Hydrolysis data are available for a number of geranyl and citronellyl esters *in vitro* in gastric juice and intestinal fluid (Grundschober, 1977; Hall, 1979), in intestinal and mucosal preparations, rat liver, and in rat and human blood. Hydrolytic activity is greatest in the liver (half-lives normally in the order of a few minutes) (Buck and Renwick, 1998). These data would support the rapid conversion of the esters to the alcohols *in vivo* in terrestrial species including humans. The presence of carboxylesterase isozymes in aquatic species (liver, whole body, etc.) and relative rates of ester hydrolysis in a variety of fish and mammals (Barron *et al.*, 1999; Li and Fan, 1997) support the conclusion that hydrolysis of esters also occurs in aquatic species. These conclusions could be further supported for example by collecting additional *in vitro* hydrolysis data with geranyl and citronellyl esters in fish and mammal liver homogenates. Hydrolysis of acetals is even more rapid than that of esters. *In vitro* experiments on simple aliphatic acetals could be supplemented with hydrolysis data in rat and fish tissues.

Following hydrolysis of the esters, the resulting primary monoterpene alcohols such as geraniol, nerol, rhodinol and citronellol undergo alcohol oxidation to yield the corresponding aldehyde (Diliberto *et al.*, 1990; Ishida *et al.*, 1989). These reactions are catalyzed by alcohol dehydrogenase and other high capacity oxidative enzymes. Also, these terpenes are subject to CYP450 -catalyzed omega-oxidation of the alkyl substituents to yield 8-hydroxy derivatives that undergo subsequent functional group oxidation to yield polar dicarboxylic acid derivatives (Hildebrandt's acid)(Parke and Rahman, 1969; Boyer and Petersen, 1990; Chadha and Madyastha, 1982). To a minor extent, hydration and selective hydrogenation also occur (Figure 2). These polar metabolites are rapidly excreted primarily in the urine of animals. Alternately, the corresponding carboxylic acids formed by oxidation of the alcohol function may enter the *beta*-oxidation pathway and eventually undergo cleavage to yield shorter chain carboxylic acids that are completely metabolized to carbon dioxide and water in the fatty acid pathway and tricarboxylic acid cycle. The enzyme systems available to catalyze these reactions have also been characterized in fish and other aquatic species supporting the conclusion that the esters and acetals are hydrolyzed to alcohols and aldehydes in aquatic species and then oxidized to even more polar products that are not bioaccumulative.

Figure 2

Metabolism of Nerol, Geraniol, and Citral in Rats



Physiochemical Properties

Although all physiochemical properties are to be provided in REACH, selected key physical properties discussed here include vapor pressure and log Kow. Values of vapor pressure are on the same order of magnitude for the alcohols, aldehydes, acetals and for formate esters and an order of magnitude greater than those for other higher MW esters.

Although key physiochemical properties of esters and acetals, such as vapor pressure and log Kow, show consistent increases with increasing molecular weight of the non-terpene carboxylic acid or alcohol component, respectively, data supporting ready hydrolysis argues that esters and acetals in the group are present *in vivo* as the corresponding monoterpene alcohols and aldehydes. Therefore, although differences in physiochemical properties exist between the esters and alcohols in this group, metabolic fate and toxic potential of the esters and alcohols are similar.

Although model predictions of physiochemical properties can be used, the collection of experimental log Kow and water solubility data for a representative aldehyde and saturated alcohol (for example tetrahydrogeraniol) would provide a more comprehensive evaluation of log Kow and solubility within the group and would allow better extrapolation of experimental ecotoxicity data to physiochemical-based model data.

Categories for Environmental Endpoints

The application of grouping concepts to the formation of chemical categories for environmental endpoints for fragrance materials can be performed by reviewing the physical-chemical properties, metabolic pathways and ecotoxicological mode of action. For example, trends for fate parameters can be established following consideration of the octanol-water partitioning coefficient and water solubility as well as an understanding of the metabolism in organisms of interest (e.g., fish for bioaccumulation). Mode of action (Verhaar et al. 1992; Russom et al. 1997) can be useful in assigning sub-categories (e.g., polar versus non-polar narcotics) to better establish trends in the hazard data. These concepts can be extended to the review of related UVCBs using their major constituents as markers.

Environmental fate

Biodegradation data for unpublished reports using standardized OECD guideline protocols clearly demonstrate that the esters, alcohols, acetals and aldehydes are readily and ultimately biodegradable, although some reduced biodegradability was noted for the acetals. Static fugacity model-based calculations for persistence in the environment show differences among members of the group. The significance of these calculations must be evaluated in the context that the substances in this chemical group are also products of plant biosynthesis and are, therefore, ubiquitous in the environment. Experimentally, members of the group have been shown to be readily and/or ultimately biodegradable, and the remainder would be expected to behave similarly in the environment. These models do not account for the influence of biogenic production on partitioning in the environment nor do they account for the hydrolysis of these substances under environmental conditions. In light of the significant impact of natural production of the terpenes and hydrolytic activity, relevance of fugacity calculations for this chemical group must be carefully scrutinized.

The existing data, supplemented with established QSAR approaches, should provide justification to limit the need for further testing.

Aquatic toxicity endpoints

Experimental ecotoxicity data derived from OECD or EEC guideline studies show LC50 values in the range from 5-15 mg/l for an ester, three alcohols and an aldehyde in the group (Attachment 1). Given the volume of production, structure (alpha-beta-unsaturated aldehyde) and key metabolic role of citral in the chemical group, an OECD guideline fish acute study could be performed to better evaluate the range of acute toxicity for the different metabolic products formed from various members of the group. For aquatic invertebrates, experimental EC50 data for Daphnia with an ester, two alcohols and an acetal could be supplemented with an acute study for citral. Esters could be placed in a subcategory and additional OECD studies could be performed for low and high MW esters. Similar arguments can be made for aquatic plants.

Bioaccumulation potential from longer-term exposure of aquatic species to monoterpene substances in this group needs to be addressed in a scientifically rigorous manner. First, data on the hydrolysis of esters and acetals in aquatic species (fish liver, etc.) should be collected to show that more lipophilic members of the group are readily converted into more polar metabolites (alcohols and aldehydes) that can be further oxidized and excreted or completely metabolized. Although data exists to show that invertebrates have the enzymatic capacity to hydrolyse and oxidize these substances, additional experimental data would need to demonstrate that these biochemical changes occur upon exposure to low levels of these substances. These types of biochemical data would focus testing requirements for chronic ecotoxicity studies on those members of the group that are indicated to bioaccumulate.

Human health endpoints

Acute Toxicity

Consistent acute mammalian toxic potential via both the oral and dermal route are demonstrated by LD50 values in the range from 4300 -6300 mg/kg with a majority of values >5000 mg/kg. These data are consistent with the conclusion that esters and acetals are rapidly hydrolysed to the corresponding alcohols and aldehydes, and that there is sufficient data to support the category.

Sensitization

Some of the materials in this group, particularly the aldehydes, have the potential to cause dermal sensitization. However, they can be formulated into consumer products at safe levels. Based on the chemical, cellular and molecular understanding of dermal sensitization, it is possible to conduct an

exposure-based Quantitative Risk Assessment (QRA) to determine safe use levels of fragrance ingredients in a variety of consumer product types.

Genotoxicity

In vitro genotoxicity data must always be evaluated in the context of existing *in vivo* data and the results of two-year carcinogenicity bioassays. Only geranyl acetate shows consistent evidence of mutagenicity *in vitro*. Geranyl acetate produces an increase in mutational frequency in the mouse lymphoma forward mutation assay (MLA). Other *in vitro* assays are uniformly negative. MLA results for these types of substances are now recognized as false positives that are caused by the formation of organic acids in metabolically competent cell lines (Brusick, 1986). These acids alter cellular buffering capacity and cellular osmolality. Based on the observation that other *in vitro* assays and the two *in vivo* assays (UDS and mouse micronucleus) are negative and geranyl acetate is not carcinogenic in either rats or mice in a 2-yr bioassay, the MLA data can be confidently classified as a false positive. In a similar manner, isolated evidence of increased chromosomal aberrations for citronellal and sister chromatid exchange for citral were obtained in non-standard assays performed at longer incubation times and at near toxic concentrations (Kasamaki *et al.*, 1982; NTP, 2001). QSAR assessment could provide direction in defining boundary substances or the need to confirm via further testing. In addition, if related esters and acetals such as geranyl acetate and citral diethyl acetal can be demonstrated to hydrolyze under the *in vivo* conditions used in the micronucleus test, additional support for the low genotoxic potential of the category could be developed.

Short-term and long-term toxicity

Key repeat dose studies exist for representative esters, acetals, alcohols and aldehydes in this chemical group. 90-day and 2-yr (NTP) studies in mice and rats for geranyl acetate/citronellyl acetate, citral diethyl acetal, geraniol, citronellol, and the mixture of geranial and neral commonly recognized as citral (see Attachment 1) are available. The slightly lower NOAELs (345 vs 1000 mg/kg in the 90-day and 210 vs 2000 mg/kg) for citral versus geranyl acetate/citronellyl acetate can be understood in terms of the known palatability issue and bodyweight reduction related to administering high dietary levels of aldehydes (citral). Disregarding the bodyweight effect, the NOAELs for citral and the ester are surprising similar. Importantly, neither substance showed any significant evidence of carcinogenicity in the 2-yr assay. Key 90-day and 28-day data for a homologue of citronellal (melonal, 2,6-dimethyl-5-heptenal) provide additional data to assess the hazard potential of the category. The results of 90- and 28-day studies for citronellol, geraniol and

citral diethyl acetal confirm the toxic endpoints and potential of the category. It would be important that repeat dose histopathology data on sex organs be provided from all new studies in order to properly evaluate the results of reproductive studies with members of the chemical group and mitigate the need for additional reproductive studies (see below).

Reproductive and developmental toxicity endpoints

Current data to evaluate the reproductive toxicity potential of the chemical group consist of two studies for citral that support NOAELs in the range of 50-60 mg/kg. Other screening reproductive/developmental toxicity data for citral diethyl acetal and melonal (2,6-dimethyl-5-heptenal) support maternal NOAELs in a similar range (125-300 mg/kg). Note that repeat dose studies, both short and long-term studies, for geranyl/citronellyl acetate, melonal and citral show no evidence of changes to the reproductive organs of males or females. Studies for boundary substances (geranyl acetate and citral) could be supported, for example, by screening studies for the acetal and melonal. Such screening reproductive/developmental toxicity studies may be considered rigorous for hazard evaluation purposes, if results are consistent with those of more rigorous studies and QSAR assessment.

Data to measure developmental potential is similar to that for the reproductive endpoint. Data for two studies on citral is supported by screening data from the acetal and melanal. Developmental NOAELs from these four studies are on the same order of magnitude. QSAR and metabolic profiles may suggest the need for further testing based on identification of boundary substances

Concluding Remarks

Experimental data on the metabolic fate and toxic potential for various endpoints for representative members of the group support the following conclusions:

1) the esters and acetals in this chemical group are readily converted to the corresponding alcohols and aldehydes, respectively, in this group.
2) all substances in this group and the constituents of the naturally occurring mixture in this group participate in common metabolic pathways of detoxication.
3) the physiochemical properties of members and hydrolysis products are consistent and based on model calculations and experimental biodegradation data that indicate a lack of persistence for members of the group in the environment.

4) acute ecotoxicity data show a similar acute toxic potential for the esters, alcohols and aldehydes. Hydrolysis data in aquatic species would support the conclusion that these naturally-occurring substances do not bioaccumulate.

5) evaluation of toxicity enables clear similarities to be determined within the category. Further assessment of the data and use of QSAR will provide justification for the need to perform additional testing.

Using this approach one can identify a limited number of additional studies to strengthen the read-across structure of the chemical group hazard assessment. Data currently available present strong evidence that the hazard potential of a single member can be adequately assessed in the context of the available data for the chemical group.

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