

PSD and ACP approach to assessing the mammalian toxicity (and consumer/operator risk assessment) of two or more compounds in a pesticide product (formulation).

Introduction

A number of the terms that are used in relation to the combined toxicity of two or more chemicals have been interpreted in different ways by different people or organisations. For the purposes of this regulatory update, the term "combined toxicity or combined action" has been used to cover the toxic effects of components of a pesticide formulation or mixture acting together.

The potential for chemicals, including pesticide active substances, to exert combined toxicity other than simple additivity of effects has been acknowledged. This has been used in a positive way in the development of some therapeutic regimes and pesticide formulations (e.g. the addition of piperonyl butoxide to pyrethrin based products). In the text accompanying the All Approval Holders Letter announcing the UK review of anticholinesterase compounds it was stated under 'Phase v' that the assessment would include consideration of potential combined effects (AAHL/19/98 of September 1998). In terms of human health risk assessments for pesticides, the potential for combined action has been considered recently in the UK by a working group of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (<https://cot.food.gov.uk/>).

The Working Group on Risk Assessment of Mixtures of Pesticides and Similar Substances (WIGRAMP: <https://cot.food.gov.uk/cotwg/wigramp>) was set up at the request of the Food Standards Agency (FSA). The first meeting was held in December 2000 and a report was published in September 2002 (<https://cot.food.gov.uk/cotwg/wigramp/wigrampfinalreport>). WIGRAMP concluded that, when performing risk assessments, the default assumptions for combined toxicity should be that chemicals with different toxic actions will act independently (simple additivity of effects) and that those with the same toxic action will act with additivity of dose. The recommendations of WIGRAMP are being taken forward by a combination of research work and policy development. Two of the key outcomes will be a scientifically based systematic framework setting out when to perform combined risk assessments and the identification of groups of active substances with common mechanisms of action. While these are being developed, it is considered appropriate to outline the current approach of ACP and PSD in evaluating combined toxicity.

Basic assumptions

Two basic assumptions have been made about how components of a mixture will act together. These are simplistic, but as predicted human exposures are at the bottom end of the dose-response curve (well below NOAELs in studies), they are considered pragmatic and adequately protective:

- i. For compounds with similar toxicological actions, the assumption being used by PSD is that any combined toxicity will follow the rules of simple dose additivity:

e.g. if 5mg of compound X on its own produces a 10% reduction in a parameter and 30 mg of compound Y on its own produces a 10% reduction in the parameter; a mixture giving rise to exposures of 5 mg of X and 30 mg of Y would be expected to give rise to a reduction equivalent to that from 10 mg of X or from 60 mg of Y.

- ii. For compounds with different toxicological actions and targets it is generally assumed that actions will be independent, with simple additivity of effect:

e.g. if 5mg of compound X on its own produces a 10% reduction in parameter B and 10 mg of compound Z on its own produces a 5% increase in parameter C; a mixture giving rise to exposures of 5 mg of X and 10 mg of Z would be expected to give rise to a 10% reduction in B and a 5% increase in C.

In other circumstances (eg where two compounds cause toxicity to the same organ but by different mechanisms), the assessment of potential combined toxicity is made on an ad hoc basis.

For some active substances the precise mechanism of mammalian toxicity is not fully understood and an in depth assessment based on mechanism of action against the pest, or common target tissues / effects might be appropriate.

e.g. A product contains two components both producing relatively general toxicity. One disrupts electron transport at cytochrome a/a3 the other uncouples oxidative phosphorylation by acting as a proton ionophore. Although there is a similar action against the pest the mechanism is not identical. In mammals neither produced effects on body weight gain or body temperature – likely targets given the mechanism of action against the pest. Although both compounds produce liver toxicity at moderate doses, and hence it might be assumed they have a common mechanism of toxicity, one affects the centrilobular region and produces necrosis at high doses, the other only produces mid-zonal hypertrophy. Therefore it can be argued that at low exposure levels they will act independently.

Formulated products.

It is the responsibility of the applicant / approval holder to evaluate the toxicity of formulated products. This evaluation has been primarily for the acute toxicity, irritancy and sensitisation classification and labelling, but other aspects of formulation toxicity also need to be considered.

i. Products containing a single active substance.

Relevant application types

All applications for new products, re-registration and major formulation changes that require a review of the human health risk assessment must include a consideration of the combined toxicity of the components. For minor formulation changes and revisions not impacting on the human health risk assessment there is no need to consider combined toxicity at this time – it will be addressed at re-registration

Issues to consider

If data indicate that the toxicity of the formulated product is clearly greater than predicted, based on simple additivity of the effects of the individual components, an explanation will be required.

When determining the classification of a product using the calculation method outlined in the Dangerous Preparations Directive (1999/45/EC) and CHIP3 regulationsⁱ the default position is that the components will act with dose additivity for acute toxicity and irritancy, but act independently (ie with simple additivity of effects) for other hazards such as skin sensitisation, carcinogenicity, mutagenicity and reproductive toxicity.

For pesticide formulations, which are subject to a risk assessment, the potential for combined effects to impact on the risk assessment is an additional consideration.

If two co-formulants or a co-formulant and the active substance are known to have the same toxic mechanism of action or target tissue at low doses the potential for combined action should be addressed. It is accepted that for many co-formulants there is limited information on the toxicity following repeated exposures. However, when the toxicity is known to be similar to that of an active substance in the product, consideration should be given to potential combined toxicity other than simple additivity of effects.

A related issue for co-formulants is whether they can produce an increased systemic exposure to the active substance e.g. by increasing absorption relative to values seen with other types of vehicles. Assessments of dermal absorption routinely consider formulation type and will take into account potential effects of vehicles (see also comments on Tank mixes, below).

ii. **Products containing two or more active substances.**

Relevant application types

All applications for new products, re-registration and major formulation changes that require a review of the human health risk assessment for pesticide products based on more than one active substance must include an evaluation of the potential combined toxicity of the active substances. For minor formulation changes and revisions not impacting on the human health risk assessment there is no need to consider combined toxicity at this time – it will be addressed at re-registration

Issues to consider

In addition to the considerations described above for formulated products containing one active substance, the following should be addressed.

When a formulated product contains two or more active substances there is a need to consider the potential for them to act other than by simple additivity of effects, in a manner that might impact on the consumer and operator/ worker/ bystander risk assessments. This concept has been part of ACP considerations for a number of yearsⁱⁱ

Applicants submitting formulated products containing two or more active substances must comment on whether the mechanism of toxicity or the target tissues for the active substances are common. The effects relevant to the combined assessment are those that either drive the critical LOAELs / NOAELsⁱⁱⁱ used for setting reference doses, or are evident at dose levels in the range of^{iv} the critical LOAELs / NOAELs. If the target tissues are common, even though the mechanism is different, there should be some case-by-case consideration of combined action e.g. a formulation containing a compound that damages red blood cells combined with one that reduces the capacity to produce new red cells might produce additive effects. If there is a common link, the impact on the overall risk assessment of potential combined action will need to be considered. A number of approaches could be applicable [See Annex 1 for a worked example], including:

- a. Determine the proportion of the respective reference doses taken up by the predicted exposures to each active substance. If this is only a small proportion (e.g. <50% if there are two components; <33% for 3 etc...) then assuming simple dose additivity the risks would still be acceptable. However if exposures to each active substance represent a high proportion of the respective reference doses (e.g. 60%) then risks might not be acceptable if the sum of the individual contributions is >100% and a more detailed consideration would be needed. In performing such an assessment for new active substances, which do not yet have agreed reference values, applicants should consider the possibility of the final agreed regulatory reference doses being lower than their initial proposals.

- b. Perform a detailed consideration e.g.
- i.) If the reference doses for the active substances are based on different toxicological effects, propose effect specific reference doses and perform the determination in a) against the effect specific reference doses;
 - ii.) examine the mechanism of toxicity of the active substances, the molecular structures, target molecules / cells or tissues and present a scientifically justified case on the potential for interaction;
 - iii.) review the recommendations for use rates, timings, PPE to see if changes can be made that will lead to acceptable exposures taking the additive effects into account.
- c. If an acceptable risk assessment cannot be demonstrated by other means it might be necessary to perform additional studies to investigate potential combined effects. The studies could use the formulation as sold or simple mixtures of the components that have the potential to act in a combined manner. The studies should focus on the effects driving the risk assessment and use dose levels in the region of the NOAELs / LOAELs for the individual components. On animal welfare grounds, the feasibility of an *in vitro* approach should be considered in the first instance. If *in vivo* studies are required, these should use the minimum numbers of animals necessary to resolve the issues.

If one active substance is known to be a potent inhibitor of xenobiotic metabolising enzymes (e.g. some conazoles) the potential impact on the metabolism of other components should be considered.

iii. **Products containing synergists, agonists or herbicide safeners**

Relevant application types

All applications for new products, re-registration and major formulation changes that require a review of the human health risk assessment for pesticide products containing synergists, agonists or herbicide safeners must include an evaluation of the combined toxicity of the active substance(s) and the synergists, agonists or herbicide safeners. For minor formulation changes and revisions not impacting on the human health risk assessment there is no need to consider combined toxicity at this time – it will be addressed at re-registration

Issues to consider

In addition to the considerations described in i. & ii. above for formulated products containing one or more active substances, the following should be addressed.

Synergists, agonists and herbicide safeners are designed to be biologically active and modify the action of the active substance. In the case of synergists and agonists there is an increased action on the target pest, with the herbicide safeners there is a reduced action on the crop. The default assumption in performing human health risk assessments on formulated products containing such biologically active compounds is that the effects induced in the pest or crop (e.g. altered metabolism of the active substance) could potentially apply to human exposures. The applicant must present data or a reasoned case to address the potential impact on the human health risk assessment of co-exposure to the active substance(s) and the synergist / agonist / safener.

Residues in foodstuffs.

For formulations containing more than one active substance, where multiple residues would be predicted, the potential for interaction will be considered as described in ii. above.

Surveillance work by the Working Party on Pesticide Residues (WPPR) and its successor the Pesticide Residues Committee (PRC) has often shown the presence of multiple residues in a single sample. There is also the potential for exposure to more than one residue from different foods in the diet. This was one of the stimuli for the formation of WIGRAMP.

As a concluding part of the UK review of anticholinesterase pesticides, PSD is developing methodology for the performance of a combined assessment of residues of anti-cholinesterase pesticides. The Medical and Toxicology Panel of the ACP has been consulted on the proposed strategy. On completion, the findings of the evaluation will be made public. Similar assessments of other groups of pesticides might be performed in the future.

At present there is no need for approval holders to take any action in respect of multiple residues in foodstuffs, other than for formulations containing >1 active substance (see ii. above).

Tank mixes.

Currently the only specific restriction on tank mixing, that is triggered by potential effects on health, is that precluding the mixing of two or more anticholinesterase compounds unless expressly permitted by the conditions of approval; as laid out in Schedule 3, section 6 (1) of the COPR (amendment) regulations 1997.

Recommendation 1.27 of the WIGRAMP report specifically mentioned the need to consider tank mixes. Investigations of the patterns of tank mixing have shown that the majority of tank mixing does not involve active substances with similar toxic actions. The Medical and Toxicology Panel considered the information and concluded that the current practices of tank mixing in the UK were not likely to give rise to significant concerns for combined toxicity based on dose additivity.

On the related aspect of altered dermal penetration the Panel believed there might be an issue due to the potential for increased systemic exposure when different types of formulations are combined. Operator exposure estimates include a value for dermal penetration of the active substance from the formulation and in-use dilution. For some active substances the degree of penetration varies with the formulation type. It is thus possible that tank mixing two or more different types of formulation could result in an increase in dermal penetration relative to that from a single formulation. The greatest concern would relate to a tank mix involving a product giving a low dermal absorption (e.g. a solid containing active substance and mainly inert components such as kaolin), with one containing solvents or surfactants that could significantly enhance the penetration. An increase in dermal absorption by 5 fold could have a greater impact on the risk assessment than simple dose additivity from 2 compounds with the same mechanism of toxicity.

PSD has commissioned a research project to investigate the effects of co-formulants on the dermal penetration of a representative range of active substances.

In considering the overall risks associated with tank mixing, the Medical and Toxicology Panel noted that because tank mixing reduced the number of application operations performed there could be a reduction in the overall risks (including physical injury).

At present there is no need for approval holders to take any action in respect of tank mixing. However, if approval holders are aware of information that is pertinent to the investigation of the impact of co-formulants on dermal penetration they are invited to contact PSD.

Annex 1

A PROPOSED METHODOLOGY FOR ASSESSMENT OF OPERATOR RISK FOR PRODUCTS CONTAINING MULTIPLE ACTIVE SUBSTANCES

The ACP has for some time considered the combined toxicity of formulated products where more than one active substance is included in a single formulation.

The initial assessment approach follows the conclusions about the potential for interaction drawn by WIGRAMP. Most applications considered so far by the committee have been for products including active substances with differing modes of action. As such the conclusion has been reached that there is little potential for additivity of dose.

A recent example has highlighted the need for an agreed methodology to assess applications where there is potential for additivity of dose. This paper presents a proposed tiered approach and a worked example.

The worked example presented below focuses on operator / worker / bystander assessments but the basic principles are equally applicable to long-term (ADI / NEDI based) and acute (ARfD / NESTI based) consumer risk assessments.

Tier 1

Calculate the estimated exposure (usually systemic exposure) to each of the active substances as a fraction (or percentage) of the AOEL agreed for that substance. If the sum of the fractions is ≤ 1 (or $\leq 100\%$), exposure of the operator is acceptable.

This tier of the assessment can be completed quickly and easily from the information available fairly readily in evaluations of the active substances and using the standard exposure assessment approaches.

If the sum of the fractions > 1 a more refined assessment will be required.

Tier 2

In this case, for each active substance and exposure scenario the toxic effect of concern for the combined risk assessment must be identified. The critical NOAEL is then derived for that effect, and an effect specific AOEL is derived by the use of an appropriate assessment factor. This effect specific AOEL will always be \geq the overall AOEL for the active substance.

Estimated exposure for each active substance is then compared to the effect specific AOEL for each substance and presented as a fraction (or percentage).

If the sum of the fractions is ≤ 1 (or $\leq 100\%$), exposure of the operator is acceptable.

If the sum of the fractions > 1 exposure is not shown to be acceptable and further specific data will need to be generated to address the concern.

Tier 2 might require some re-evaluation of relevant studies where the earlier evaluation document does not enable the derivation of effect specific NOAELs. Again standard methods of estimating exposure are appropriate.

Tier 3

If estimated exposure is not shown to be acceptable at tier 2, further specific data will be required to address the risk. This might include operator monitoring data, in vitro testing of the combination, or in vivo testing of the combination.

Worked example

Background information

Data presented below are derived from a real example.

PRODUCTX contains four active substances, Substance A, Substance B, Substance C and Substance D. Consumer exposure to all active substances resulting from the use of this product is insignificant. Hence consideration of the potential for combined toxicity other than simple additivity of effect is relevant only to the risk assessment for operators and workers. (Bystander exposure is also unlikely to be significant).

Three of the active substances have the same mechanism of pesticidal action, which is suggestive of some commonality of toxicological effect.

The liver was a key target organ for all four active substances and liver effects were considered relevant for setting the short-term systemic AOEL for substances A and B. Hence there is a potential for additivity of dose on the liver being relevant for risk assessment of operators and workers exposed to PRODUCTX. However, it should be noted that much of the commonality of liver effects related to enzyme induction, which is often regarded as adaptive.

Enhancement of spontaneously occurring malformations and structural anomalies was seen after dosing with Substance B (microphthalmia, supernumerary ribs) or Substance D (including microphthalmia, cleft palate) and teratogenicity/developmental toxicity had an impact on setting of the ARfD and /or short-term systemic AOEL for these two substances. Substance B was seen to cause developmental anomalies (supernumerary ribs) only at a high oral dose level (750 mg/kg bw/day, no effects at 80 mg/kg bw/day in rats) with a LOAEL for malformations of 1000 mg/kg bw/day based on microphthalmia in rats. Substance C was a more potent developmental toxin causing effects at oral doses of 10 mg/kg bw/day (including arthrogryposis in rabbits and supernumerary ribs in rats). Hence there is a need to consider additivity of dose on malformations/structural anomalies as relevant for risk assessment of operators and workers exposed to PRODUCTX (primarily from exposure to Substances B, C and D).

Table 1 provides for each active substance the mechanism of pesticidal action and the basis for setting toxicology reference values, together with a brief outline of a few notable toxicological effects (not a comprehensive summary).

Table 1. Summary of effects of active ingredients in PRODUCTX

Active ingredient (reference)	Mechanism of pesticidal action and basis for toxicology reference dose values (mg/kg bw/day)
Substance A	<p>Mechanism of pesticidal action:</p> <p>A strobilurin fungicide that causes inhibition of mitochondrial respiration. This prevents oxidative phosphorylation and thus causes a severe reduction in the energy source (ATP) for a cell.</p> <p>Mammalian toxicity</p> <p>Liver was a key target organ (evidence of increases and decreases in hepatic activity and/or function) and included hepatocytomegaly and hepatocellular hypertrophy. Disruption of calcium and phosphorus homeostasis was an important effect in the rat. There were kidney effects at high doses.</p> <p>Short-term systemic AOEL (0.03) based on: Increased serum alkaline phosphatase in dogs (indicative of possible toxic effects on liver and/or bone).</p> <p>ADI (0.015) based on: Reduced body weight gain and increased serum alkaline phosphatase in a 12-month dog study:</p> <p>ARfD (0.3) based on: Reduced body weight gain in dogs</p>

Substance B	<p>Mechanism of pesticidal action:</p> <p>A triazole fungicide that causes fungal steroid biosynthesis inhibition (SBI) (inhibition of cytochrome-P450 dependent C-14 demethylase reaction, (DMI))</p> <p>Mammalian toxicity</p> <p>The liver and kidney were target organs. Liver effects included raised plasma ALT levels, changes in hepatic enzyme activity (generally increased) and increased liver weights; hepatocellular hypertrophy was sometimes seen. Teratogenic/developmental and reproductive effects were seen at a high dose level in the presence of maternal toxicity.</p> <p>Short-term systemic AOEL (0.25 based on: Liver effects (increased weight, enzyme activity and histopathological findings) in mice and liver effects (increased weight and serum ALT) and kidney effects (histopathology) in dogs</p> <p>ADI (0.05) based on Crystalline material in urine sediment, increased severity of chronic progressive nephropathy and transitional cell hyperplasia in the urinary bladder. Increased liver weights and increased incidences of centrilobular hepatocellular hypertrophy with cytoplasmic change and eosinophilic/clear cell foci with cytoplasmic change.</p> <p>ARfD (0.5) based on: Reduced body weight gain or body weight loss in pregnant rats and rabbits. Account was also taken of microphthalmia at a high dose level (LOAEL 1000 mg/kg bw/day, NOAEL 500 mg/kg bw/day) in rats.</p>
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Substance C	<p>Mechanism of pesticidal action: SBI (Class I, DMI).</p> <p>A triazole fungicide that causes fungal steroid biosynthesis inhibition (inhibition of cytochrome-P450 dependent C-14 demethylase reaction).</p> <p>Mammalian toxicity</p> <p>The liver a main target organ. Liver effects included hepatic enzyme induction and histopathological changes (including necrosis). Teratogenic/developmental and reproductive effects (including reduced litter size) were seen.</p> <p>Short-term and long-term systemic AOEL (0.01) based on: developmental effects in rabbits (increased incidence of malformations including arthrogyrosis).</p> <p>ADI (0.01) based on Liver histopathology and reduced weight gain in a rat chronic toxicity/carcinogenicity study,</p> <p>ARfD (0.01) based on: Developmental effects in rabbits (increased incidence of malformations including arthrogyrosis).</p>
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Substance D	<p>Mechanism of pesticidal action:</p> <p>A triazole fungicide that causes fungal steroid biosynthesis inhibition (inhibition of cytochrome-P450 dependent C-14 demethylase reaction)</p> <p>Mammalian toxicity</p> <p>The liver, adrenals and red blood cells were target organs. Liver effects included increased serum ALT, liver enzyme induction, increased weight, fatty change, enlarged hepatocytes, bile duct proliferation, and (in mice) hepatocellular tumours. Teratogenicity in rats, mice and rabbits seen at maternally toxic doses. Incidence of stillbirths was possibly increased in multigeneration study.</p> <p>Short-term systemic AOEL (0.06) based on: NOEL for teratogenicity of 30 mg/kg bw/day in mice and rabbits (LOAEL 100 mg/kg bw/day), with application of 500 fold safety factor. No correction for oral absorption is needed</p> <p>ADI (0.03) based on 12 month dog study: Lipid vacuolation of the adrenals, lenticular lesions and increased liver enzyme activity.</p> <p>ARfD (0.06) based on: NOEL for teratogenicity of 30 mg/kg bw/day in mice and rabbits (LOAEL 100 mg/kg bw/day), with application of 500 fold safety factor.</p>
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Tier 1.**Assessment of the significance of the commonality of toxic effects for the overall risk assessment of PRODUCTX**

In order to assess the significance of this commonality of key toxicological effects for the risk assessment of operators and workers exposed to PRODUCTX, short-term AOELs are compared with relevant exposure levels in Table 2.

Table 2: Short-term AOELs compared with exposure levels for operators and workers following use of the PRODUCTX

Substance	Short-term systemic AOEL (mg/kg bw/day)	Predicted systemic exposure (mg/kg bw/day)	Predicted exposure as % of short-term systemic AOEL
Operators			
Substance A	0.03	0.011	37%
Substance B	0.25	0.0866*	35%*
Substance C	0.01	0.0039	39%
Substance D	0.06	0.0115*	19%*
		Total = 0.113	Total = 130%
Workers			
Substance A	0.03	0.0078	26%
Substance B	0.25	0.028*	11%*
Substance C	0.01	0.00062	6%
Substance D	0.06	0.00402*	7%*
		Total = 0.04	Total = 50%

(*) These values are based on the default assumption that dermal penetration is 100%.

The potentially additive dose of all 4 substances is of possible concern.

For operators, Table 2 shows that for each substance predicted systemic exposure is <40% of the short-term systemic AOEL and that when these percentages are summed the total is 130%. An acceptable level of exposure has not been demonstrated at this tier. For workers, the situation is more favourable, with overall totals for such a comparison of 50%.

Tier 2

a). Liver effects

In order to provide an indication of effect specific AOELs, NOAELs/NOELS for liver effects are presented in Table 3.

Table 3 **NOAELs/NOELs for liver effects and derivation of effect specific AOELs**

Substance	NOAEL/NOEL for liver effects (mg/kg bw/day)	LOAEL/LOEL for liver effects (mg/kg bw/day)	Comments
Substance A	NOAEL = 3	LOAEL = 8	NOAEL based on increased serum alkaline phosphatase activity, after 87 days in 1 y dog study, which could be indicative of effects on liver or bone. NOAEL also supported by increased serum alkaline phosphatase activity and reduced hepatic function (reduced serum protein and albumin) at 24-25 mg/kg bw/day in a 90-day dog study. Effect specific AOEL = 0.03
Substance B	NOAEL/NOEL = 100	LOAEL/LOEL = 300	The NOAEL in the 90d dog study was 100 mg/kg bw/day based on liver effects at 300 mg/kg bw/day (including increased weight, increased ALT but no histopathological changes and no clear substance-related effects serum alkaline phosphatase). In a 90 day mouse study there was a lower liver NOAEL than the 90 day dog study. The NOAEL was 25 mg/kg bw/day based on effects at 100 mg/kg bw/day ie increased liver weight, increased microsomal enzyme activity and histopathological changes consistent with enzyme induction (hypertrophy, cytoplasmic change, fatty change). Effect specific AOEL = 1
Substance C	NOAEL = 7.8	LOAEL = 38	NOAEL based on increased liver enzyme activities, weights and histopathology indicative of enzyme induction (ground glass cytoplasm) a 90-day dog study Effect specific AOEL = 0.08
Substance D	NOEL = 8.3	LOEL = c 40	NOEL based on slight increase in alkaline phosphatase and N-demethylase activity in a dog 90 day study. Effect specific AOEL = 0.08

Table 4. **Comparison of exposure with effect specific AOELs for liver effects**

Substance	Effect specific AOEL (mg/kg bw/day)	Predicted systemic exposure (mg/kg bw/day)	Predicted exposure as % of effect specific systemic AOEL
Operators			
Substance A	0.03	0.011	37%
Substance B	1	0.0866*	9%
Substance C	0.08	0.0039	5%
Substance D	0.08	0.0115*	14%
		Total = 0.113	Total = 65%

b) Malformations/structural anomalies

In the case of malformations/structural anomalies, the potentially additive dose of substance B, substance C and substance D are of possible concern.

In order to derive effect specific AOELs, NOAELs for malformations (excluding supernumery ribs) in oral studies are compared in Table 5.

Table 5 **Comparison of NOAEL for malformations in oral (gavage) studies* and derivation of effect specific AOELs**

Substance	NOAEL for malformations (mg/kg bw/day)	LOAEL for malformations (mg/kg bw/day)	Comments
Substance A			No malformations seen – not included further in this assessment
Substance B	500 (rats)	1000 (rats)	NOAEL (rats) based on microphthalmia No malformations seen in rabbits Effect specific AOEL = 0.5 (Assessment factor 1000)
Substance C	2(rabbits) 10 (rats)	10 (rabbits) 30 (rats)	NOAEL (rabbits) based on arthrogryposis and multiple abnormalities NOAEL (rats) based on forelimb dysplasia, and fetuses with multiple abnormalities. Effect specific AOEL = 0.01 (Assessment factor 200)
Substance D	30 (rabbits) 30(mice) 60 (rats)	100 (rabbits) 100 (mice) 100 (rats)	NOAEL (rabbits) based on peromelia and other effects NOAEL (mice) based several effects including cleft palate NOAEL (rats) based on microphthalmia, anophthalmia and other effects. Effect specific AOEL = 0.06 (assessment factor 1000)

(*) For the purposes of this comparison, supernumery ribs are not regarded as malformations

Table 6 **Comparison of exposure with effect specific AOELs (for malformations)**

Substance	Effect specific AOEL (mg/kg bw/day)	Predicted systemic exposure (mg/kg bw/day)	Predicted exposure as % of effect specific systemic AOEL
Operators			
Substance B	0.5	0.0866*	17%
Substance C	0.01	0.0039	39%
Substance D	0.06	0.0115*	19%*
		Total = 0.113	Total =75 %

c) Conclusions from tier 2

The potentially additive dose from the 4 active substances in PRODUCTX does not present a significant health risk to workers and operators as far as effects on liver are concerned.

Although 3 of the 4 active substances in PRODUCTX cause malformations/structural anomalies, the potentially additive dose does not present a significant health risk to operators or workers.

Tier 3

In this example had there been a need to consider further specific data requirements standard dermal absorption data could have been used to refine the exposure estimates. The applicant could opt to provide such data instead of a tier 2 assessment in this case.

ⁱ The Chemicals (Hazard Information and Packaging for Supply) Regulations 2002, Statutory Instrument 2002 No. 1689

ⁱⁱ Page 11 of the ACP report for 2000 assessment of triticonazole; Page 12 of the ACP report for 2001 assessment of flufenacet.

ⁱⁱⁱ Low Observed Adverse Effect Levels or No Observed Adverse Effect Levels - derived from toxicity studies

^{iv} For severe / irreversible effects that would merit an additional assessment factor this range could extend for an order of magnitude.