Technical Notes for Guidance

Revision of Chapter 6.2 (Common Principles and Practical Procedures for the Authorisation and Registration of Products) of the TNsG on Product Evaluation, and a revision of Chapter 10[1] (Assessment for the potential for resistance to the active substance) of the TNsG on Annex I Inclusion.

This document was endorsed at the 33rd meeting of representatives of Members States Competent Authorities for the implementation of Directive 98/8/EC concerning the placing of biocidal products on the market (13-15 May 2009).

[1] In this document the structure (numbering of chapters and sub-chapters) of Chapter 6 of the TNsG on Product Evaluation is followed.

CHAPTER 6 ASSESSMENT OF OTHER UNACCEPTABLE EFFECTS

6.1 GENERAL INTRODUCTION

6.1.1 Background

The evaluation of unacceptable risks to humans, animals and the environment (including to non-target organisms (e.g. beneficial insects) and the atmosphere (e.g. ozone depletion)) are dealt with in Chapters 3-5. This chapter provides guidance for the assessment of other effects which contribute to the overall performance of the product but which are not directly linked to its intrinsic properties or efficacy.

In accordance with Article 5 (1) (b) of the Directive, the competent authority must assess the potential unacceptable effects of the product on target organisms, such as unacceptable resistance, and any unacceptable suffering caused by use against vertebrates. Annex VI also requires competent authorities to evaluate the possibility of any other unacceptable effects occurring if there are indications that they may do so.

6.1.2 Objective of the guidance

This chapter, used together with expert scientific judgment, gives guidance for competent authorities on the evaluation of unacceptable effects data so they can decide how these will influence the authorisation.

The range of potential unacceptable effects is very broad and there are no internationally agreed guidelines for their assessment. In addition, relevant information can be complex, and may be obtained from a variety of sources. Consequently the guidance is of a general nature and information for each product must be assessed on a case by case basis. Detailed information about specific properties and effects is available in a variety of reference texts (e.g. Buckle & Smith, 1994).

Resistance, humaneness and 'other' effects are dealt with in three separate sections, and particular attention is paid to the types of data which might be available and the decision making process. **In all cases it is the responsibility of the applicant to provide all relevant information for the competent authority, in a structured and readily accessible format.** The guidance is valid for all countries in the European Union. However, situations within certain territories may vary due to different working practices, environmental conditions, and the relevance and breeding biology of the target species.

6.2 RESISTANCE

6.2.1 Introduction and Definitions

Annex IIA of the Directive requires information on the occurrence and possible development of resistance, and appropriate resistance management strategies, for chemical active substances. Annex IIB of the Directive requires information on any known limitations on efficacy of the biocidal product including resistance.

The evaluation of resistance must be done on a case-by-case basis taking into account the possible development of resistance (see chapter 6.2.3.3). A number of factors need to be considered:

The term resistance refers to a genetically inherited characteristic, which cannot be acquired during the lifetime of the organism. **Resistance** can be defined as a heritable decrease in susceptibility or a lack of susceptibility of an organism to a particular treatment with an agent under a particular set of conditions. The term 'resistance' is often used loosely, and incorrectly, to explain treatment failure which may be attributed to inadequate treatment, behavioural changes of the target pest, target pest tolerance or other contributory factors.

One has to distinguish between **acquired resistance**, i.e. when the decreased susceptibility or insusceptibility is the result of genetic changes due to mutation or the acquisition of appropriate genetic material (e.g. plasmid coded resistance genes in bacteria), and **intrinsic resistance**, an already existing, inherent property of a certain species resulting in low or insusceptibility. Another distinction can be made between **stable** and **transient resistance,** considering reversibility of the resistance. From this point of view, transient resistance results from a temporary adaptation induced by the changes of the environment (stress).

An important phenomenon is the occurrence of **cross resistance**: wherever a species develops resistance to a particular active substance, it may also be resistant to other active substances to which they have not previously been exposed, due to (i) chemical similarity of the compound having the same mode of action, (ii) in case of overlapping targets or (iii) low specificity of the resistance mechanism. Laboratory studies have shown the possibility of cross-resistance between biocides and antibiotics, and between biocides themselves.

Different from cross resistance is **co-resistance**. Co-resistance refers to the presence of several resistance mechanisms in the same organism (also designated as multi-resistance). The corresponding genes are adjacent (physically linked) and expressed in a coordinated fashion.

The **level of resistance** of a particular genetic strain can be quantified in laboratory studies by the resistance factor (or ratio), which is the number of times the amount of biocide given to a resistant strain has to be increased above the normal dose to achieve the same effect as that dose in the normal strain.

For antibacterial biocides, the nature and the level of resistance of a particular microbial strain can be assessed in laboratory studies by using the Minimum Inhibitory concentration (MIC) (or Minimum Bactericidal Concentration / Minimal Fungicidal Concentration), the changes of the bactericidal kinetics, and the molecular biology techniques to detect the genes responsible of the resistance.

The level of resistance, its geographical spread and frequency of occurrence can all change with time for any one biocide (indeed there can be a wide variation in resistance levels across a single country). It should be noted that some biocides will continue to have a commercial usefulness even at reduced levels of efficacy towards a particular target species.

Intrinsic resistance should be detected during efficacy testing of biocidal compounds and could therefore be regarded as not being a subject for an assessment for the

potential for resistance. Intrinsic resistance may, however, remain undetected, if test measurements are not sufficiently related to the treatment conditions that prevail under practical conditions, or when certain factors, that render insusceptibility, are simply unknown.

Unlike intrinsic resistance, that appears unexpectedly solely when the underlying conditions or factors leading to a decreased susceptibility were formerly unknown, acquired resistance in fact turns up newly in a population of a pest organism. Since acquired resistance develops after a certain period, it cannot be detected by efficacy testing of a new active substance or biocidal product in advance.

Resistance Mechanisms

Three main types of resistance mechanisms are presently known:

1. **Detoxification** of active compounds by the production of degrading or modifying enzymes.

2. **Target-site alteration**, i.e. modification of the target molecule that is "attacked" by the active compound.

3. **Reduced uptake** into the body or **decreased penetration** mainly of antimicrobial compounds by impermeability and efflux pumps - passive, which involves alterations of outer membrane structure, decreasing the rate of entry of active compounds and over expression of efflux pumps that exports the active compound outside the cell. In this way organisms can become resistant to many different compound classes (cross resistance).

In higher organisms like insects or rodents, changes in susceptibility are based almost exclusively on acquired resistance through genetic changes.

Treatment failure as a result of **behavioural changes** of the target pest can be displayed in a number of ways, such as bait preference and neophobia. Behavioural changes do not involve actual systemic resistance to a biocide's action, and it can be reversible.

An example of bait preference is the altering of feeding habits from protein to carbohydrate baits. Obviously if bait preference changes or is different depending on the change in the life cycle of the pest, then the biocidal product will have varying degrees of efficacy.

Neophobia or "new object reaction" is exhibited by some rodent species, and refers to individuals who avoid a new object (such as a bait) placed in the environment until they become used to it. As a result the individual may only take a small, sub-lethal amounts of bait, and may consequently avoid the bait if it learns to associate it with an unpleasant response.

Some of these behavioural aspects can be anticipated and tested through experimental design when biocidal products are being developed but others can only be overcome by the expert use of the biocide by trained professional operators.

Tolerance can be defined as the ability of an organism to withstand the effect of a normally lethal dose of a biocide by ingestion of increasingly large sub-lethal doses over a short period of time.

Tolerance is different from resistance because if the normal lethal dose is administered in single dose the individual will die (resistant individuals will not).

For bacteria, the term tolerance is frequently used for specific mechanisms leading to a maintaining of the inhibitory of growth activity but a loss of bactericidal efficiency i.e. for ß-lactams against some *Staphylococcus aureus* strains.

It can be seen from the above points that the potential for actual resistance must be identified to establish that a resistant management strategy is required.

Where relevant the Competent Authority should evaluate the extent and nature of existing resistance to an active substance by the target organism, and anticipate its development, so that a balanced Annex I inclusion decision can be made.

6.2.2 Types and availability of data

Whilst data should be relevant to the target species, requirements must be flexible because of the variable nature of resistance. Evidence of resistance may come from:

- laboratory studies specifically addressing resistance (including determination of mutation frequency, simulated use and dose-response tests), e.g. efficacy studies on strains which are known to be resistant to the active substance. For vertebrates there may be specific, non-lethal methods of resistance assessment, such as blood clotting tests for rodenticide anticoagulants; or

- field studies (in which data are generated using the product in the actual service conditions and in the manner described on the product label). Field observations may also be provided as additional evidence (however, see section 6.2.3.1).

- Resistance data will usually be available for existing active substances following review for Annex I inclusion, but there are unlikely to be any data for new active substances. However the Competent Authority may be able to make a decision based on relevant information on products containing an active substance from the same chemical class with a similar mode of action against similar target organisms.

If valid data are available in connection with resistances to existing active substances, these should be added or references made to the relevant publications. These data will usually be available for existing active substances following review for Annex I/IA inclusion, but it is unlikely that there will be any data for new active substances. However, the competent authority may be able to make a decision based on relevant information on products containing an active substance from the same chemical class with a similar mode of action. Similarly, data are not necessarily required for every product because an extrapolation may be possible from data on similar products containing the same active substance.

6.2.3 Evaluation

6.2.3.1 General principles

The applicant's data submission should include, where relevant, all information necessary to allow a reasonable evaluation of target organism resistance to the biocidal product at the recommended dose/application rate, when used in accordance with the label instructions. Data on the active substance itself will have been considered at the Annex I/IA inclusion, and must not be re-interpreted. Where product data are provided, the competent authority should perform the evaluation with regard to:

- test objective;
- spectrum in reference to the claim;
- study content and methodology (including use of controls and reference products, test procedures, results and analysis, etc.);
- acceptability of the method;
- robustness;
- quality assurance;
- completeness; and
- adequacy (i.e. its reliability and relevance to the proposed use of the candidate product)
- field data from any source should be taken into account.

Expert judgement is needed for proper interpretation of resistance data. For example, data generated on laboratory strains may not be reliably extrapolated to wild individuals in the field situation. In addition, field observations should be viewed with caution. For example, persistent infestations are often caused by re-invasion from untreated surroundings or poor application techniques rather than resistance. Apparent resistance may also be caused by behavioural factors, such as neophobia (as is often the case for rats). For this reason, the competent authority will need evidence to show that other possible causes of treatment failure have been excluded. Corroborating data would usually also be needed from laboratory tests on captured specimens.

Conclusions about the performance of the product should usually be valid for all areas of the Member State in which it is to be authorised, and all conditions under which its use is proposed. However, where there are pockets of resistance within a Member State’s territory, the competent authority should decide whether continued use of the product can be allowed elsewhere within the territory (e.g. it may be possible to contain the resistant pockets by a suitable management strategy (see 6.2.3.4)). Decisions may also need to be made regarding read-across of resistance data for similar species, also from other genus or families in the case of microorganisms, especially where the intention is to extend the label claim.

6.2.3.2 Cross-resistance

The problem of cross-resistance also needs to be addressed for products. This will be necessary when the active substance has a similar mode of action or mechanism of resistance (i.e. porins modification in Gram negative bacteria) or belongs to a particular chemical class, which is known to cause resistance problems in particular situations (e.g. pyrethroids used to control fly problems in intensive animal units). Information on known resistance problems with related active substances should be provided in meeting the Annex IIA data requirements for the active substance. In such cases, the competent authority should ensure that adequate data on the activity of the product against these resistant strains have been provided.

6.2.3.3 Development of resistance

As well as assessing the immediate likelihood of resistance for the product, the competent authority must, where relevant, evaluate the possibility of the development of resistance to the active substance by the target organism. This will normally be considered at the Annex I/IA inclusion, but it may be appropriate to consider this for particular products as well. However, it is likely that resistance development will only become evident as the product is used. The ability of laboratory tests to predict such development can be relatively low, because they often show only the symptoms of resistance rather than the underlying cause or because resistance has not been established in the genetic pool within the relatively short duration of the test. Factors that may promote the development of resistance are related to the mode of action of the active substance, the lifestyle of the target organism and the proposed use pattern of the biocidal product. Examples of such factors include:

- active substances that act by a “one site” (as opposed to a “multi-site”) mechanism;
- active substances able to induce a high frequency of mutation;
- target organisms with rapid breeding cycles (i.e. many generations per year);
- pest infestations that are confined in some way (where resistant individuals are unable to disperse and so remain localised);
- use of the biocide over large areas and/or for long periods with frequent application rates (creating a continual evolutionary selection pressure on the target population);
- use of the biocide over biofilm;
- use of a number of biocidal products against the same pest which contain either the same active substance or active substances with similar modes of action;
- use of active substances that expose “multi-generations” of the target organism as opposed to single generations to one application is more liable to cause resistance.

As regards **acquired resistance**, the two basic factors that affect the probability of the emergence of new resistance traits are related to the mode of action of the active substance and to the biology of the target organism:

(i) **The specificity of the biocide mechanism** (the likelihood of resistance development generally increases with the specificity of the biocide mode of action), and

(ii) **The reproduction rate of the target organisms** (the likelihood of resistance development increases with the turnover rate of generations and the population size).

In addition, a number of important conditions and factors that have to be considered are related to the use pattern of the biocidal product:

(iii) **Site of application** - confined, closed areas (e.g. laboratory equipment) where a thorough elimination of pests are intended (no or very low survival rate) are less prone to resistance development than open, unconfined areas, where the number of individuals can only be reduced to an acceptable level.

(iv) **Controllability of exposure**, controllable use ensures the appropriate and regular way of application. Uncontrolled use of the biocide in an inappropriate way – too low doses and/or too short time – may not only lead to the survival of target organisms with an inducible intrinsic (cross-) resistance, but may as well lead to the enrichment of genotypes with an elevated tolerance towards the given agent.

(v)**Use of the biocide** – is it intended to use the product over large areas and/or for long periods with frequent application rates? Such treatments create a continuous evolutionary selection pressure on the target population. It is widely agreed that the most efficient way to delay the development of drug resistance remains the reduction of selection pressure, i.e. decreasing the number of treatments. Are there biocide residues on surfaces? Is there some interference between the biocide and the soil surfaces (decreasing the efficacy by lessening the effective concentration)?

6.2.3.4 Resistance management strategies

Where resistance is considered likely to be a problem for use of a particular active substance at the Annex I/IA inclusion, an overall management strategy should be implemented in order to help delay or reduce the likelihood of resistance development, and minimise any consequences. The competent authority must evaluate the proposed use of the product in the light of any strategy recommended at the time of the Annex I/IA inclusion, and where necessary ensure that the applicant submits a supplementary management strategy for particular products (such a strategy may be based on the principles of integrated pest control, but should be distinguished from actions which are tailored to control site-specific resistant infestations).

The competent authority must assess these proposals to determine their acceptability, and whether they are appropriate to the use of the product, on a case by case basis. For example, a strategy which aims to limit the number of resistant individuals rather than eradicate them may be suitable for housefly control in intensive animal units but would not be acceptable for the control of cockroaches in food-handling premises.

<u>What is a resistance management strategy?</u>

The immediate aim of resistance management is to prevent or retard the development of resistance to a given biocidal active substance while permitting its continued use, as far as possible without being counterproductive. The ultimate aim is to reduce or

eliminate the adverse consequences of resistance. The central concept is that this can be done more effectively and cost-efficiently by integrated, cohesive and systematic action than by the normal, default option in which all the parties involved improvise their own ways of addressing the problem. In this sense the approach has much in common with IPM (integrated pest management), and uses the same wide range of techniques.

Where relevant, contact should be sought with the International Resistance Action Committees (RACs)[2]

Because the emergence of resistant individuals is a natural phenomenon and therefore unavoidable, the only means to manage resistance development is to prevent or to delay the dissemination of resistant target organisms (or the resistance genes) by appropriate measures, that match the above mentioned fixed conditions and factors, **and** that are comprised of a specific mode of pest treatment and of surveillance of resistance spread.

The appropriate measures and procedures that would be adequate for biocides do not in general differ from those that have been suggested for pesticide use (EU Directive 91/414/EEC) and that have been outlined and discussed in detail in a number of contributions published by the Resistance Action Committees (RACs). The main objective and purpose of these measures can be summarized as:

- minimize the selection pressure as far as possible, but
- take care not to apply sub lethal doses allowing better adapted individuals to survive.

Without question, the deployment of a suitable range of alternative active substances is necessary for the management of resistance and to prolong the useful lifespan of those active substances to which resistance has become a problem. The following practices are among a number of the more feasible options available to retard the onset of resistance, where resistance is identified as a significant problem:

- the incorporation of appropriate label warnings or provision of other labelling advice, for example not using the biocidal active substance in isolation. Consideration of application with one or more biocides of a different type (biocidal diversity), or as one component in a rotation of different treatments.
- Restriction of the number of treatments applied, and application only when strictly necessary. Special requirements could be defined for disinfectants and general biocidal products (main group 1), as related resistance is affected by several factors such as concentration, temperature, type and time of application.
- Use of non-chemical control techniques, where available.

[2] The RACs give advice on the use of pesticides (www.irac-online.org; www.rrac.info; www.hracglobal.com; www.frac.info). It will often be easy to broaden their field of work to biocides, such as in the cases of fungicides and insecticides which are used both in pesticidal and biocidal applications.

- A switch to another biocidal active substance to which resistance rarely or never develops (or alternance).

- Ensuring complete eradication with a specific biocide and resuming the current treatment (or association).

- Maintaining uncontrolled, susceptible populations in refugia (in isolated areas) from which emigration can occur.

- specific conditions of authorisation, e.g. restrictions on the use of the active substance(s) in a particular situation or geographical area.

Note: These are general measurements on how to manage resistance. Supplementary strategies may be required later for individual products (see TNsG for product evaluation for further information).

Resistance Monitoring

When resistance has been detected and a resistance management strategy instituted, monitoring is necessary to determine its effectiveness. Some form of surveillance, such as questionnaire surveys, sampling for mutations known to confer resistance (e.g. in rodent populations), investigation of reports of inefficiency, or some other form of feedback reports, may also help towards early detection of new cases of resistance.

6.2.4 Examples

Resistance should be considered for all product types where there is a possibility of its development (this will usually be identified at the Annex I/IA inclusion for the active substance). The following list gives some examples of product types with well-known resistance problems, but it is not exhaustive.

Product type 14: Rodenticides: e.g. resistance of rats to first and second generation anti-coagulant rodenticides.

Product type 18: Insecticides, acaricides and products to control other arthropods: e.g. resistance of houseflies to synthetic pyrethroid insecticides in intensive animal units.

In addition, biocidal products for control of micro-organisms may be prone to resistance problems. Relevant product types include disinfectants (Product types 1-5), preservatives for liquid cooling and processing systems (Product type 11), slimicides (Product type 12) and metal-working fluids (Product type 13).

6.2.5 Decision making

Having evaluated all the available data, the competent authority must determine whether resistance to the biocidal product is likely now or in the future, the significance of this in relation to performance, and possible management strategies to control the problem and minimise any consequences. Based on this assessment the competent authority will decide which of the following will apply:

- authorisation/registration can be granted without specific conditions, because the data demonstrate a level of resistance which will have little effect on product performance, and the potential for any further development of resistance is low;

- the level of resistance or its development may affect product performance, but the biocidal product can be authorised/registered subject to specific conditions (e.g. a management strategy) or for a specific time period (followed by a review);

- a decision on authorisation/registration cannot be given until additional data/information are available to resolve a particular point or item of concern; or

- the biocidal product cannot be authorised/registered because product performance will be unacceptably affected by resistance, and/or the potential for the development of resistance is of concern and the proposed management strategy is considered inadequate to control it.

This decision must be a reasoned balance between the benefits of using a product and the loss of performance caused by any resistance problems (real or potential), taking into account the availability of other control methods and the implications of the loss of the product through refusal of authorisation (the wider the diversity of active substances that are available, the easier it will be to control future resistance problems).