Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Spain for the existing active substance

RAPESEED OIL

of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, part 3, B.7

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ANNEX B

RAPESEED OIL

B - 7 : RESIDUE DATA

B.7 Metabolism and residues data

Introduction

Rapeseed oil is a naturally-occurring oil. It is a mixture of esters (triglycerides) of different fatty acids. The main fatty acids in Rapeseed oil are oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$). Fatty acids are an integral part of the cell membranes of every living organism. They also occur as food substrate in the form of their triglycerides, i.e. fats and oils. Linoleic and linolenic acid are essential fatty acids in humans.

Rapeseed oil is used as a contact insecticide/acaricide against spider mites, mealy bugs and scales in ornamentals (greenhouse and field) and orchards. In orchards it is used at the start of the vegetation period before the development of fruits (**Table 7.5-1**).

Rapeseed oil is also used as a food commodity with an average consumption of about 7.3 g/person/day in the European diet[1].

B.7.1 Stability of residues

B.7.1.1 Stability of residues during storage of samples

B.7.1.2 Stability of residues in sample extracts

Notifier statement: Rapeseed oil is a food grade substance. Maximum residue values that could theoretically arise from application in harvestable crops (orchards) are negligible compared to the daily consumption (see **B.7.6.1**) and intended at the start of the vegetation period when no contact with fruits is expected. As a consequence, no residue trials were performed. A study on the stability of residues in samples or sample extracts is therefore also not required.

RMS assessment: RMS considers that the reported literature regarding plant metabolism is not conclusive about the behaviour of rapeseed oil when is applied on the plant and some information is needed before a conclusion about the residue definition is set. Although fatty acids occur naturally in plants, some data is needed to confirm that “not naturally occurrence fatty acids” or undesirable compounds are not found in the plant as a consequence of the application of rapeseed oil. (see **B.7.2.1**). Depending on the results, studies on the stability of residues may be required.

[1] “Global Environment Monitoring System (GEMS)/Food Regional Diets”, compiled by the WHO

B.7.2 Metabolism, distribution an expression of residues in plants

B.7.2.1 In plants, in at least three crops representative of the different categories of crop (root vegetables; leafy crops; fruit crops; pulses and oilseeds; cereals)

Notifier statement: No specific metabolism studies with Rapeseed oil were performed since triglycerids and fatty acids occur naturally in plants, are degraded rapidly and their degradation pathways are well known.

Like all vegetable oils, Rapeseed oil is a mixture of several different fatty acids bound by ester linkages to the glycerol molecule. There does not seem to be any system in the formation of the glyceryl esters. It appears that the several fatty acids present in the oil are randomly distributed in ester linkages to the glyceryl residues. The C_{18} fatty acids, which are the major fatty acids in Rapeseed oil, are commonly found in many plant seeds and other plant tissues. **Table 7.2.1-1** lists the fatty acids occurring in seeds which are used to make vegetable oils (**Ting, 1982; IIA 6.2.1/01**). These seeds show a wide variation in contents of C_{18} fatty acids.

Table 7.2.1-1: Examples of contents of C_{18} fatty acids in some seeds commonly used as sources of vegetable oils

Seed	Palmitic acid ($C_{16:0}$) [%]	Stearic acid ($C_{18:0}$) [%]	Oleic acid ($C_{18:1}$) [%]	Linoleic acid ($C_{18:2}$) [%]
Olive	6	4	83	7
Sunflower	4	3	34	59
Cotton	22	2	31	45
Peanut	6	4	61	22

Fatty acids are a natural component of living plant tissue and provide three major functions:

First, they are used as building blocks of more complex compounds in the plant. Most fats, oils and phospholipids are directly derived from the fatty acid substrate. The fatty acids do not usually occur in the free form but are covalently bound in different classes of lipids. The main fatty acid of Rapeseed oil, $C_{18:1}$, exists in a wide range of plants.

The second main function of the fatty acids is to provide the structural integrity of the cellular membranes. In the membranes, the fatty acids occur as phospholipids consisting of phosphoglycerides which contain two fatty acid molecules esterified to the first and second hydroxyl group of glycerol.

The phospholipids are formed into bilayers with the polar heads oriented outward and the non-polar tails oriented inward. These lipid bilayers make up the structure of the cellular membranes and are meshed with proteins to form the typical lipo-protein cell membrane.

The <u>third</u> main function of the fatty acids is to provide high energy food sources. The fatty acids are used as food substrate for virtually all life forms including bacteria, fungi, plants, and animals. On oxidation the triacylglycerols provide over twice much energy per gram as carbohydrates. In humans the unsaturated C_{18} fatty acids linoleic acid and linolenic acid cannot be synthesized but must be ingested for normal nutrition. These fatty acids are essential for proper growth and development.

Degradation of triacylglycerols starts with the removal of the fatty acid residues from glycerol by acyl hydrolases. This degradation can be very rapid in plant tissue.

Acyl hydrolases are common to all plants and are very active during phases of the plant's growth cycle or during times of stress. For example the enzyme activity greatly increases during: seed germination, due to metabolism of the seed oils; fruit ripening, due to loss of membranes; senescence of leaves (including leaf decay in the soil); storage of fruits and vegetables; and infection by pathogens or damage by external agents.

After triacylglycerol is broken down into fatty acids and glycerol, fatty acids are degraded further by various oxidative pathways. **Table 7.2.1-2**, (**Galliard, 1978; IIA 6.2.1/02**), lists the major processes for enzymatic oxidation of fatty acids in plants.

Table 7.2.1-2: Major processes of enzymatic oxidation of fatty acids in plants

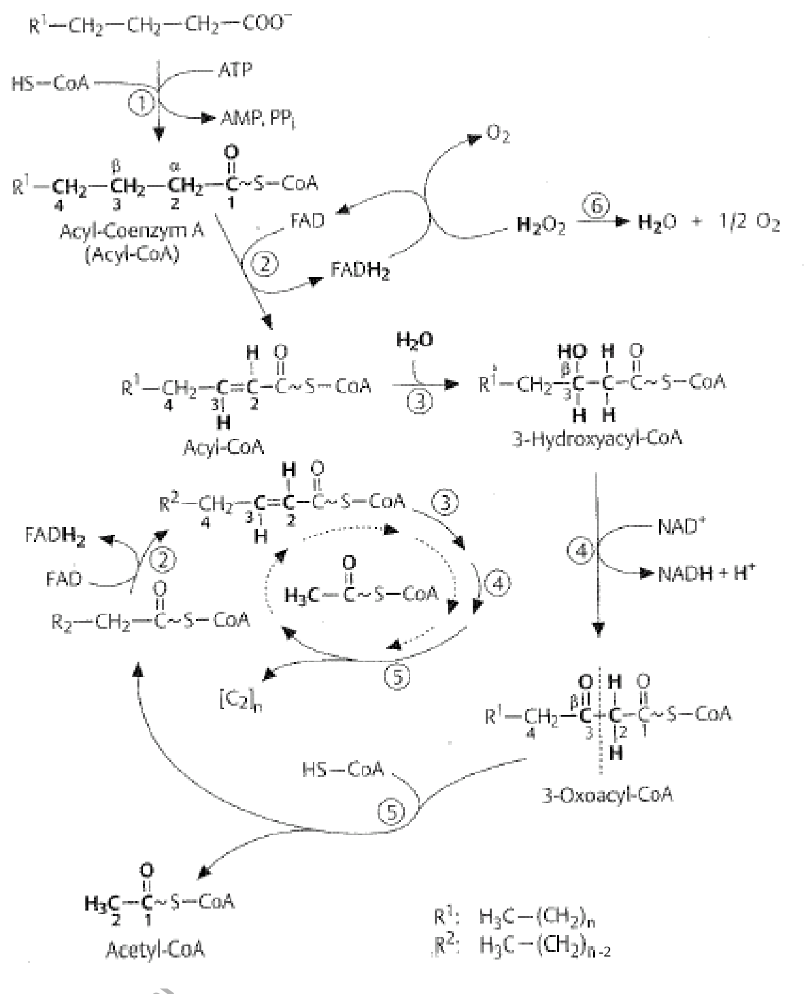
	Substrate		**Cofactors, etc.**	**Initial Products**
	free acid	**thio ester**		
α-oxidation	+ +		a) O_2 b) $O_2 + NAD^+$	2-D-hydroxy acid Cn-1 fatty acid + CO_2
β-oxidation		+	NAD^+, flavo-protein	Acetyl CoA
ω-oxidation	+		a) O_2, NADPH b) $O_2 + NADP^+$	ω-hydroxy acids Dicarboxylic acid
in-chain hydroxylation		+	O_2, NADPH	In chain hydroxy acids
in-chain epoxidation		+		Epoxy acids
peroxidation	+		O_2, (Fe-enzyme)	Fatty acid hydro-peroxides

The enzymatic pathway of <u>α-oxidation</u> proceeds with free fatty acids. Thus it can play an important part in the further degradation of free fatty acids released from glycerolipids by acyl hydrolases or exogenously applied fatty acids. In young leaves, (**Galliard, 1978; IIA 6.2.1/02**) reported that fatty acids are more rapidly utilised by α-oxidation than by β-oxidation. The metabolic pathway of α-oxidation involves a 2-D-hydroperoxy acid intermediate which may be either reduced to the 2-D-hydroxy acid (as found in cerebrosides) or decarboxylated to the C_{n-1} aldehyde, which may be subsequently oxidized to the C_{n-1} acid to continue the α-oxidation cycle (**Hitchcock and Nichols 1971: IIA 6.2.1/03**).

<u>β-Oxidation</u> is the principal mechanism for the degradation of the fatty acids into CO_2 and water in plants and animals. It is associated with the mitochondria and the main respiration functions of living cells. The sequence of this oxidation is shown in **Figure 7.2.1-1**.

The carboxyl group of the fatty acid reacts with ATP to form an energy-rich adenylic derivative. This derivative then reacts with coenzyme-A (CoA) to form acyl CoA. Acyl CoA is then oxidized between the number 2 and 3 carbons to form a double bond. The electron acceptor in this oxidation is a flavin. Water then adds across the double bond to form a hydroxyl group on the beta-carbon. Oxidation leads to double bond oxygen on carbon No. 3. The derivative then reacts with CoA, generating an acetyl CoA unit and a C_{n-2}-CoA derivative. The acetyl CoA is further metabolized by the citric acid cycle to CO_2 or by the glyoxylate cycle to release CO_2 and sucrose. The C_{n-2}-CoA unit proceeds through sequential cycles of similar oxidations until it forms acetyl-CoA.

Fig. 7.2.1-1: β-Oxidation of fatty acids

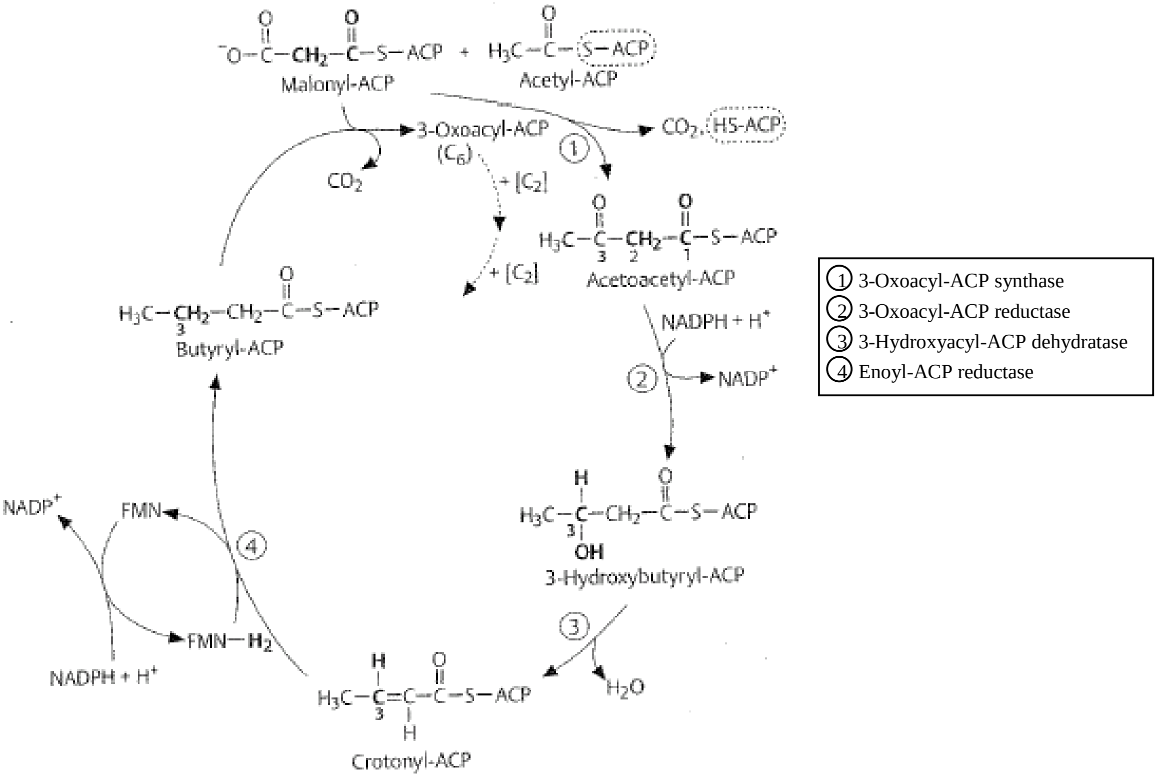


1. Acyl-CoA synthetase
2. Acyl-CoA dehydrogenase
3. Enoyl-CoA hydratase
4. Hydroxyacyl-CoA dehydrogenase
5. Acyl-CoA acyltransferase (ß-Ketothiolase)
6. Catalase

Besides degradation into successively smaller fatty acids by loss of 2-carbon units, fatty acids are also interconverted during biosynthesis. Synthesis takes place in the cytosol up to a chain length of C_{16}. The overall reaction in this fatty acid synthetase system involves the acyl carrier proteins acetyl ACP and malonyl ACP (**Figure 7.2.1-2**).

The single molecule of acetyl ACP serves as the starter unit. Chain growth occurs with the starting acyl residue and grows by successive additions of 2-carbon units, each derived from malonyl ACP, proceeding toward the carboxyl end of the fatty acid. After the initial acetyl group, each successive 2-carbon unit originates from the 2-carbon atoms of the malonyl group which are nearest the ACP. Simultaneously, the third carbon atom of malonyl ACP, i.e. the unesterified carboxyl group, is lost as CO_2.

Fig. 7.2.1-2: Biosynthesis of fatty acids



Fatty acids longer than C_{16} can be formed through the action of fatty acid elongation systems which occur in the endoplasmic reticulum and the mitochondria. Double bonds can be introduced into the molecules by oxidative reactions catalyzed by fatty acid-CoA.

Further transformations can occur by <u>ω-oxidation</u> and in-chain hydroxylation to form the complex polymers of cutin or suberin. Reaction can also lead to the triacylglycerols and phospholipids of the membranes.

Fatty acids are absorbed by the leaf where they are used in the normal metabolic processes of the plant. Bacteria that inhabit the leaf surface will interact with the exogenous fatty acid substrate and begin to utilise it as a nutrient source using the pathways described above. Ionized fatty acid and non-dissociated fatty acid molecules will penetrate through the lipophilic layer of the cuticle and enter the cytoplasm of the cells (**Rivera and Penner 1979; IIA 6.2.1/04**). Once inside the cells, the lipids will be metabolized similar to the endogenous fatty acids and either broken down for energy or used to synthesize the more complex triacylglycerols or phospholipids. Plants rapidly convert the free fatty acids since, in this form, they are phytotoxic and will cause cellular damage and possibly necrosis, if allowed to accumulate.

Tso and his colleagues (**Tso et al. 1975, IIA 6.2.1/05; Tso and Chu 1977, IIA 6.2.1/06**) undertook detailed studies on the fate of ^{14}C-labelled dodecanoic acid, dodecyl alcohol and methyl dodecanate on tobacco leaves. Tso et al. (1975) applied 960 mg of $^{14}C_{12}$ methyl ester or 750 mg of $^{14}C_{12}$ methyl alcohol to 3-5 tobacco plants after topping. Plants were harvested 2 weeks after spraying and air-cured. Recovery of ^{14}C-activity was very low after air-curing with an average recovery of < 0.02%. Residues corresponding to 1.45, 1.73 and 0.75 ppm were recovered from Maryland, Burley and Bright tobacco, respectively, after application of the labelled ester derivative. Application of $^{14}C_{12}$ methyl alcohol resulted in labelled residues equivalent to 1.35, 0.99 and 1.01 ppm for the same tobacco species. These amounts were compared to 7000 ppm naturally occurring fatty compounds in tobacco.

Tso and Chu (1977) reported that when C_{12} fatty acid was sprayed on tobacco plants, most of the residue remained in the acid fraction but when dodecyl alcohol was used, 9.7 to 24.8% was converted to the acid fraction and 7.4 to 14.8% was converted to the ester fraction. With the methyl ester, even more was converted with 54 to 77% of the residue being recovered in the acid fraction and 12.6 to 22.7% in the alcohol fraction, 16-144 hours after spraying. There was a significant loss of ^{14}C activity during the test period of 144 hours in all trials.

A clear example of the rapid interconversion of exogenously applied fatty acids is demonstrated by the research of **Murphy and Stumpf, 1980, IIA 6.2.1/07** using labelled oleic and linoleic acids on cucumber seedlings. When cucumber cotyledons were incubated for 3-32 hours in a solution of [^{14}C]oleic acid in ethylene glycol monoethyl ether, oleic acid was esterified to complex lipids at a rapid rate for the first 6 hours of incubation and at a constant but slower rate for the next 26 hours. The greatest increase in labelled lipid was in phosphatidyl choline. Phosphatidylethanolamine and diacylglycerol showed similar kinetics. The principal end product of the conversions was triacylglycerol. Labelled linoleic acid was converted even faster than oleic acid and its esterification into complex lipids was virtually complete in 6 hours after the lipid was applied. Phosphatidyl choline was the main lipid acceptor for the labelled linoleic acid. Diacylglycerols were also transiently labelled but monogalactosyl diacylglycerol and triacylglycerols were the sites of ^{14}C accumulation.

RMS assessment: The notifier did not provide any study on the metabolism of rapeseed oil in plant material but much literature about the metabolism of triacylglycerols and fatty acids in plants. The degradation of triacylglycerols (components of rapeseed oil) involves the hydrolysis of triacylglycerols to glycerol and fatty acids following further fatty acids degradation by various oxidative pathways. These processes, as it is compiled in the literature, take place in plant tissues and inside the cell (mitochondria, cytosol, endoplasmic reticulum…).

The hydrolysis is catalysed by acyl hydrolases, which are common to all plants but these enzymes are activated in very special circumstances, during phases of the plant's growth cycle or during times of stress, for example during seed germination (due to metabolism of the seed oils), fruit ripening (due to loss of membranes), senescence of leaves (including leaf decay in the soil), storage of fruits and vegetables and infection by pathogens or damage by external agents. Nevertheless it was not reported the ability of triacylglycerols to penetrate into the plant cells when they are applied on the plant (the reference **Rivera and Penner 1979; IIA 6.2.1/04** does not address this issue) and it was assumed that bacteria that inhabit the leaf surface will interact with the exogenous glycerides, and fatty acids will be absorbed by the leaf and used in the normal metabolic processes of the plant. A reference was reported about the metabolic behaviour of C_{18} fatty acids in cucumber cotyledons that involves also the formation of triacylglycerol but none study was reported on the behaviour of triacylglycerols, the main components of rapeseed oil.

Fatty acids occur naturally in plants. Each of the lipid classes in membranes has a variety of fatty acids associated with it, the distribution of which includes the following major fatty acids: myristic (14:0), palmitic (60:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic acid (18:3). Therefore the notifier argues that it is not possible to distinguish between the applied Rapeseed oil and naturally occurring plant fatty acids. But RMS considers that other not naturally occurring plant fatty acids could be identified if treated plants are compared to control plants.

RMS considers that the reported literature is not conclusive about the behaviour of rapeseed oil when is applied on the plant and, taking into account the arguments given above, some information is needed before a conclusion about the residue definition is set.. Although fatty acids occur naturally in plants, some data is needed to confirm that “not naturally occurrence fatty acids” or undesirable compounds are not found in the plant as a consequence of the application of rapeseed oil.

In addition, the intended uses of Rapeseed oil are: Ornamentals, woody ornamentals and orchards. The use “*orchards*” is very wide and the notifier should specify the use in the table of intended uses under GAPs in order to carry on the studies and make the evaluation.

B.7.2.2 Metabolism, distribution an expression of residue in livestock

Notifier statement: No specific metabolism studies with Rapeseed oil were performed in livestock, since the fatty acids comprised in Rapeseed oil occur naturally in animal feed, are degraded rapidly and their degradation pathway in plants and animals is well known. Due to the rapid degradation and conversion into other compounds by the crop, it is not expected that residues of Rapeseed oil can be distinguished from the plants own fatty acids in fruits at harvest.

The first step in ingestion of fats by animals is to break the compounds into molecules small enough to pass the cell membranes of the tissues lining the gastrointestinal tract. About 40-50% of the ingested fat is reduced to fatty acids and glycerol and a similar portion is reduced to monoacylglycerols with short chain fatty acids. The rest is absorbed as di- and triacylglycerols. Digestion of fat usually begins in the stomach where food stuffs that have natural emulsifiers are broken into fatty acids and glycerol. Fatty acids released at this stage may be exchanged in the stomach or intestine with fatty acids of other triacylglycerols, to modify the composition of the ingested fat. Long chain fatty acids are more likely to be reincorporated into triacylglycerols than are short chain fatty acids, as the synthesis and hydrolysis of fat occurs simultaneously (**Guthrie, 1975; IIA 6.2.2/01**).

Once in the small intestine, bile salts secreted by the gall bladder break the larger fat molecules into smaller particles. In the finely divided state, the fats are acted upon by the pancreatic lipases and intestinal lipases. As previously discussed, the enzymatic activity releases the fatty acids from the glycerol core. The fat´s breakdown progresses from triacyl- to diacyl- and monoacylglycerols with the fatty acid in the middle position. Consequently, at any particular time, fat exists as a mixture of tri-, di-, or monoacylglycerols, glycerol and fatty acids, with about 90% being changed to the more soluble monoacylglycerols, glycerol and fatty acids before absorption (**Guthrie, 1975; IIA 6.2.2/01**).

The digested or divided fat molecules are taken up from the gastrointestinal tract as separate molecules of fatty acids and glycerol, and as mono-, di- and triacylglycerols. About 30% of the free fatty acids with less than 12 carbon atoms and glycerol, combine with bile salts. These allow the lipids to be released into the intestinal cells from which they, in turn, are released into the lymphal and portal systems. Once within the mucosal cells of the intestinal lining, many of the remaining free fatty acids with more than 12 carbons in their chain, unite with a different molecule of glycerol to form fats as mono, di, or triacylglycerols. About 70% of the absorbed fat is re-synthesized immediately to form triacylglycerols. These enter the lacteals, fat collecting ducts for lymph, and are carried through the lymphatic system. The fat molecules absorbed through the lacteals are transported as microscopic fat particles called chylomicrons. These are stabilized combinations of 99% fat with 1% protein. Fat collected in the lymphatic ducts empties into the general circulation in the neck region and is then circulated to all body cells.

Lipids that have been removed from the blood as it passes through the liver and other tissues are hydrolyzed or split into fatty acids and glycerol by cellular lipases. The fatty acids reappear in the blood as free fatty acid components of lipoproteins, the soluble combination of a fat with a protein. Fat is removed from the blood lipoproteins by various tissues and meets one of two fates. It can be broken down through β-oxidation by the enzymatic pathways, to form carbon dioxide, water and energy as described above for plants (α-oxidation is used for methyl-branched fatty acids), or it may be stored in the fat depots of each cell or in special adipose cells, for future use as a source of energy.

Table 7.2.2-1 summarises the compositions of such depot fats in different species of livestock as stated by **Sinclair, 1964; IIA 6.2.2/02**. Some fat is used by the mammary gland during lactation in the production of milk and some is used in the synthesis of other substances (e.g. phospholipids of cell membranes, enzymes). When the body has need for energy reserves, the fat stored as triacylglycerols, is hydrolyzed by lipases within the cells, to fatty acids and glycerol. The fatty acids leave the cell, become bound to the protein albumin in the bloodstream and are carried to the tissues requiring energy. The glycerol core of the stored triglycerides is returned to the liver for metabolism.

Table 7.2.2-1: Fatty acid contents of depot fats of livestock species

	Depot fat [%]				
	Cow	**Sheep**	**Horse**	**Pig**	**Chicken**
$C_{14:0}$ and below	3	3	5	1	1
$C_{16:0}$ (Palmitic acid)	29	25	26	30	25
$C_{18:0}$ (Stearic acid)	21	28	5	16	4
$C_{20:0}$ and above	1	0	traces	0	0
$C_{16:1}$ (Palmitoleic acid)	3	1	7	3	7
$C_{18:1}$ (Oleic acid)	41	37	34	41	43
$C_{18:2}$ (Linoleic acid)	2	5	5	7	18
$C_{18:3}$ (Linolenic acid)	0	0	16	0	0
$C_{20:4}$ and above	traces	1	2	2	1

RMS assessment: The notifier did not provide any specific study on the metabolism of rapeseed oil in livestock but literature about the metabolism of fats was submitted. The fatty acids comprised in Rapeseed oil occur naturally in animal feed and their metabolism in animals is well known. Furthermore, Rapeseed oil is also used as a food commodity and therefore metabolism studies on rapeseed oil itself are not needed. Depending on the residue definition for plant material and the clarification on the intended uses, more information may be needed.

B.7.3 Definition of the residue

B.7.3.1 Proposed maximum residue levels (MRLs) and residue definition.

Notifier statement: Since the fatty acids of Rapeseed oil occur naturally in plants and since they are rapidly degraded or transformed into other plant compounds so that they are soon indistinguishable from plant endogenous fatty acids at harvest, **it is proposed not to set a residue definition.**

No MRLs are proposed since the fatty acids in Rapeseed oil are indistinguishable from the plant's own fatty acids at harvest and it is therefore not possible to determine residue levels in harvestable goods. Furthermore, Rapeseed oil is a natural food commodity. The application amount in orchards is negligible compared to the daily consumption of this oil in Europe (refer to the European diet[2]) (see **B.7.6.1**).

RMS assessment: Rapeseed oil is intended to be applied long before the development of fruits in orchards and therefore residues is not likely to be present at time of harvest in the fruit. A very worst estimation of the exposure due to the use as plant protection product was compared to the exposure due to consumption of Rapeseed oil as a food commodity assuming that all the active substance applied

[2] "Global Environment Monitoring System (GEMS)/Food Regional Diets", compiled by the WHO

would be uptake by the fruit and there is not degradation. The content of rapeseed oil in fruits would result in negligible values compared to the average consumption of this commodity.

Nevertheless, RMS considers that the reported literature is not conclusive about the behaviour of rapeseed oil when is applied on the plant and some information is needed before a conclusion about the residue definition is set.. Although fatty acids occur naturally in plants, some data is needed to confirm that "not naturally occurrence fatty acids" or undesirable compounds are not found in the plant as a consequence of the application of rapeseed oil.

In addition, the intended uses of Rapeseed oil are: Ornamentals, woody ornamentals and orchards. The use "*orchards*" is very wide and the notifier should specify the use in the table of intended uses under GAPs in order to carry on the studies and make the evaluation.

RMS will do a definitive assessment on the residue definition when more information is available

B.7.4 Use pattern.

The intended uses of Rapeseed oil are: Ornamentals, woody ornamentals and orchards.

According to the information provided in the Dossier (Documemt D-2: Authorized and Actual Uses and Documemt C: Labels and leaflets) orchards, in which the use of formulation NEU 1160 I is authorized in Europe are: Pome fruit, stone fruit, berries except strawberries.

The use "*orchards*" is very wide and the notifier should specify the use in the table of intended uses under GAPs in order to make the evaluation. With the information provided in the dossier the evaluation has been made for the use in apples.

B.7.5 Identification of critical GAPs

The Good agricultural practice (GAP) proposed for NEU 1160 I (883 g/L Rapeseed oil) are summarised in table 7.5-1.

RMS assessment: The use "*orchards*" is very wide and the notifier should specify the use in the table of intended uses under GAPs in order to make the evaluation. In addition, the application rate for orchards of **8.83 kg as/ha and m crown height** is not accepted. A range of the application per ha must be specified, independently of the height of the plant.

With the provided information in the dossier the evaluation has been made for the use in apples and the application rate of 8.83 kg as/ha and a 3 m high tree.

Table 7.5-1: Good agricultural practice (GAP) proposed for NEU 1160 I (883 g/L Rapeseed oil)

Crop and/ or situation / Country	Product name	Field, glasshouse or indoor use	Pests or group of pest controlled	Formulation: Type	Formulation: Conc. of as (g/L)	Application: Method kind	Application: Growth stage & season	Application: Number per growing season (max)	Application: Interval between applications	Application rate per treatment: kg as/hL	Application rate per treatment: Water (L/ha)	Application rate per treatment: kg as/ha	PHI (days)	Remarks
Europe Representative use: Ornamentals Further intended uses: -	NEU 1160 I	Glasshouse (professional and home garden use)	Spider mites, mealy bugs, scales	EC	883	Knapsack sprayer and hand sprayer	When infestation is visible	3	7 days	1.766	2000 - 4000	35.32-70.64 (40-80 L* product/ha)	-	Effect: killing of adults
Europe Representative use: Orchards Further intended uses:	NEU 1160 I	Field (professional and home garden use)	Eggs of spider mites	EC	883	Knapsack sprayer, motor sprayer, hand sprayer	Start of vegetation up to mouse ear stage, or bud swelling up to bud break	1	-	1.766	500 per m crown height	8.83 per m crown height (10 L product/ha and m crown height)	-	Effect: suppression of winter stages
Europe Representative use: Woody ornamentals Further intended uses: -	NEU 1160 I	Field (professional and home garden use)	Eggs of spider mites	EC	883	Knapsack sprayer, motor sprayer; hand sprayer	Start of vegetation up to bud break	1	-	1.766	600-1200	10.596-21.192 (12-24 L** product/ha)	-	Effect: suppression of winter stages

* Plant height < 50 cm: 40 L product/ha (2000 L water/ha), 50-125 cm: 60 L product /ha (3000 L water/ha), > 125 cm: 80 L product /ha (4000 L water/ha)

**Plant height < 50 cm: 12 L product/ha (600 L water/ha), 50-125 cm: 18 L product /ha (900 L water/ha), > 125 cm: 24 L product /ha (1200 L water/ha)

B.7.6 Residues resulting from supervised trials

B.7.6.1 Residue trials

Notifier statement: Since Rapeseed oil is to be applied long before the development of fruits in orchards and since the components of Rapeseed oil are rapidly degraded or converted into other compounds by the plant, it is not expected that residues can be found in/on the fruits at harvest. Furthermore, as fatty acids occur naturally in plants it is not possible to distinguish between the applied Rapeseed oil and naturally occurring plant fatty acids. Residue trials are therefore considered not relevant.

Moreover, Rapeseed oil is a food commodity and the application amount of 8.83 kg a.s./ha and m crown height is negligible compared to the daily consumption of this commodity of up to 7.3 g/person/day in the European diet[3], when calculating the maximum possible residues directly after application. For instance, apples were cultivated in France on a total area of 48 400 ha with a total yield of 1 863 500 t apples in 2004[4], giving an average yield of 38.5 t/ha. Rapeseed oil is applied with a maximum of 26.5 kg a.s./ha assuming a crown height of 3 m. Assuming as a very worst case estimate that all the applied Rapeseed oil is taken up completely by the plant and accumulated exclusively in the fruits without any degradation or other losses between application and harvest, this amounts to 688 mg Rapeseed oil/kg apple. This would contribute to only 11% of the normal fat content of apples of 6 g/kg (**Watt and Merrill, 1963, IIA 6.3.1/01**). With a daily consumption of 40 g apples/person/day, this would mean an additional 0.027 g Rapeseed oil/person/day in the consumption of the European diet.

RMS assessment: Residues trials were not performed. The extent of the exposure due to the use as plant protection product was compared to the exposure due to consumption of Rapeseed oil as a food commodity. A very worst case, assuming that all the active substance applied would be uptake by the fruit, was considered. Nevertheless RMS considers that there is not homogeneity of data used for calculation:

Apples were used for the calculation. The intended uses under GAPs are "*orchards*" that is very wide and the notifier should specify the use in order to make the evaluation.

The production and cultivated area of apples in France were used for the estimation of the treated fruit per ha. But the production in Europe (or the worst case) must be used if the European diet is used for the evaluation.

[3] "Global Environment Monitoring System (GEMS)/Food Regional Diets", compiled by the WHO
[4] AGRESTE (Statistique Agricole Annuelle, Conjoncture), SNM.
Internet website: www.agreste.agriculture.gouv.fr

The application rate of 8.83 kg as/ha and m crown height, and a tree height of 3 m were used for the estimation of the amount of residue of rapeseed oil in the fruit (mg Rapeseed oil/kg apple). This application rate is not accepted. A range of the application per ha must be specified, independently of the height of the plant.

A daily consumption of Rapeseed oil of up to 7.3 g/person/day in the European diet was used. According to the new 13 GEMS/Food Consumption Cluster Diets[5], three zones for diets in Europe are identified: North (13.7 g/person/day), Centre (10.0 g/person/day) and South (0.7 g/person/day). It would be preferred to use the critical value.

A daily consumption of 40 g apples/person/day was used for the estimation. The critical consumption in the individual European diets, as it is compiled by EFSA, would be more accuracy (UK children consumption of 20.72 g/Kg/day = 180 g/person/day).

Overall conclusion: Rapeseed oil is intended to be applied long before the development of fruits in orchards and therefore residues is not likely to be present at time of harvest in the fruit. Nevertheless, considering that it is also used as a food commodity and assuming no degradation, a very worst estimation of the exposure due to the use as plant protection product was compared to the exposure due to consumption of Rapeseed oil as a food commodity, assuming that all the active substance applied would be uptake by the fruit and there is not degradation. Although RMS considers that there is not homogeneity of data used for calculation, the residue contents in fruits would result in negligible values compared to the average consumption of this commodity.

Nevertheless more information about the residue, the intended uses and rate of application is needed before a definite conclusion can be achieved.

B.7.7 Effects of industrial processing and/or household preparation

Notifier statement: Since Rapeseed oil is to be applied long before the development of fruits in orchards and since the components of Rapeseed oil are rapidly degraded or converted into other compounds by the plant, it is expected that residues of Rapeseed oil have been metabolised to CO_2 and water or are indistinguishable from fatty acids occurring naturally in the plant.

Moreover, Rapeseed oil is a food commodity and the application amount of 8.83 kg a.s./ha and m crown height forms only negligible residues compared to the daily consumption of this commodity in the European diet (refer **B.7.6.1**). Studies on the effect of industrial processing and/or household preparation on the nature and level of residues are therefore considered not relevant.

[5] www.who.int/foodsafety/chem/ClusterDietsAug06.xls

RMS assessment: Rapeseed oil is intended to be applied long before the development of fruits in orchards and therefore residues is not likely to be present at time of harvest in the fruit, nevertheless more information about the residue from metabolism studies, the intended uses and rate of application is needed before a conclusion can be achieved.

B.7.8 Livestock feeding studies

Livestock feeding studies were considered not relevant. No specific metabolism studies with Rapeseed oil were performed in livestock, since the fatty acids comprised in Rapeseed oil occur naturally in animal feed and their metabolism in animals is well known. Furthermore, Rapeseed oil is also used as a food commodity. But depending on the residue definition for plant material and the clarification on the intended uses, more information may be needed.

B.7.9 Residues in succeeding or rotational crops

Rapeseed oil is intended for the use in orchards, and in ornamentals (greenhouse and field). These crops are normally not followed by succeeding crops. Furthermore, Rapeseed oil will be degraded rapidly by soil micro-organisms and the fatty acids contained in Rapeseed oil occur naturally in the soil, resulting from degradation of organic substances or formed by micro-organisms (**see B.8**). It is therefore not expected that Rapeseed oil will have any adverse effects on succeeding crops.

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods in the case of post-harvest uses

B.7.10.1 Pre-harvest interval (in days) for each relevant crop

Notifier statement: The waiting period is not relevant in ornamentals as they are not harvested for food or feed (waiting period proposal "N"). Furthermore, it is also not relevant in orchards, since Rapeseed oil is a natural food commodity composed of natural fatty acids, which are indistinguishable from the plants own fatty acids at harvest.

RMS assessment: Rapeseed oil is intended to be applied long before the development of fruits in orchards and therefore residues is not likely to be present at time of harvest in the fruit, nevertheless more information about the residue definition for plant material and clarification on the intended uses are needed before a conclusion can be achieved.

B.7.10.2 Re-entry period (in days) for livestock, to areas to be grazed

A re-entry period is considered not relevant, since Rapeseed oil is a natural oil which is also used as a food commodity

B.7.10.3 Re-entry period (in hours or days) for man to crops, buildings or spaces treated

A re-entry period is considered not relevant, since Rapeseed oil is a natural oil which is also used as a food commodity. There is no toxicological concern of man getting in contact with this oil.

B.7.10.4 Withholding period (in days) for animal feeding stuffs

A withholding period for animal feeding stuffs is considered not relevant, since Rapeseed oil is a natural oil which is also used as a food commodity.

B.7.10.5 Waiting period (in days) between last application and sowing or planting the crop to be protected

Rapeseed oil is intended against insects and mites on ornamentals and fruit trees. It is not intended for the use before sowing or planting the crop to be protected. A waiting period is therefore not relevant.

B.7.10.6 Waiting period (in days) between application and handling treated products

Rapeseed oil is a natural oil which is also used as a food commodity. There is no toxicological concern of man getting in contact with this oil.

B.7.10.7 Waiting period (in days) between last application and sowing or planting succeeding crops

Rapeseed oil is intended for application in permanent cultures which are normally not followed by succeeding crops. Furthermore, the fatty acids contained in Rapeseed oil degrade rapidly (refer to **B.8**) and are natural components of the soil. Thus, a waiting period between application and sowing or planting succeeding crops is considered not relevant.

B.7.11 Community MRLs and MRLs in EU Member States

Up to now, no MRLs have been established for Rapeseed oil by EU or European Member State.

B.7.12 Proposed EU MRLs and justification for the acceptability of those MRLs

B.7.12.1 Proposed maximum residue levels (MRLs) and residue definition

Notifier statement: Since the fatty acids of Rapeseed oil occur naturally in plants and since they are rapidly degraded or transformed into other plant compounds so that they are soon indistinguishable from plant endogenous fatty acids at harvest, **it is proposed not to set a residue definition.**

No MRLs are proposed since the fatty acids in Rapeseed oil are indistinguishable from the plant's own fatty acids at harvest and it is therefore not possible to determine residue levels in harvestable goods. Furthermore, Rapeseed oil is a natural food commodity. The application amount in orchards is negligible compared to the daily consumption of this oil in Europe (refer to the European diet[6]) (see **B.7.6.1**).

RMS assessment: Rapeseed oil is intended to be applied long before the development of fruits in orchards and therefore residues is not likely to be present at time of harvest in the fruit. Nevertheless a very worst estimation of the exposure due to the use as plant protection product was compared to the exposure due to consumption of Rapeseed oil as a food commodity assuming that all the active substance applied would be uptake by the fruit and there is not degradation. Although RMS considers that there is not homogeneity of data used for calculation, the content of rapeseed oil in fruits would result in negligible values compared to the average consumption of this commodity.

Nevertheless aditional data on plant metabolism were required to confirm that “not naturally occurrence fatty acids” or undesirable compounds are not found in the plant as a consequence of the application of rapeseed oil. RMS considers that the reported literature is not conclusive about the behaviour of rapeseed oil when is applied on the plant (see **B.7.2.2**). In addition the use “*orchards*” is very wide and the notifier should specify the use in the table of intended uses under GAPs in order to make an evaluation.

RMS will do a definitive assessment on the residue definition when more information is available.

Residue definition for products of animal origin was considered not relevant. No specific metabolism studies with Rapeseed oil were performed in livestock, since the fatty acids comprised in Rapeseed oil occur naturally in animal feed and their metabolism in animals is well known. Furthermore, Rapeseed oil is also used as a food commodity. But depending on the residue definition for plant material and the clarification on the intended uses, more information may be needed

B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues.

No EU Import tolerances exist for Rapeseed oil.

B.7.14 Basis for differences if any, in conclusions reached having regard to established or proposed CAC MRLs

Not applicable.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means

The calculation of TMDI, NEDI and NESTI are considered not relevant, since Rapeseed oil is a food commodity and therefore no MRLs are proposed. Furthermore, as the application amount of Rapeseed oil for the use as an insecticide/acaricide is negligible compared to the daily consumption of this food commodity and application in harvestable crops is intended before the development of fruits, no

[6] “Global Environment Monitoring System (GEMS)/Food Regional Diets”, compiled by the WHO

increase in consumption of Rapeseed oil due to the use as an insecticide/acaricide is expected (see **B.7.6.1**). Nevertheless more information about the residue definition for plant material and clarification on the intended uses are needed before a conclusion can be achieved

B.7.16 Summary and evaluation of residue behaviour

Rapeseed oil is a contact insecticide/acaricide used against spider mites, scales and mealy bugs in ornamentals (field and greenhouse) and orchards. The use "*orchards*" is very wide and the notifier should specify the use in the table of intended uses under GAPs in order to make the evaluation. In addition, the application rate for orchards of **8.83 kg as/ha and m crown height** is not accepted. A range of the application per ha must be specified, independently of the height of the plant. With the information provided in the dossier the evaluation has been made for the use in apples and the application rate of 8.83 kg as/ha and a 3 m high tree.

The notifier did not provide any study on the metabolism of rapeseed oil in plant material but much literature about the metabolism of triacylglycerols and fatty acids in plants. Plants will either degrade the components of this oil to provide energy for other metabolism processes, or they will use the fatty acids to synthesize phospholipids, other fatty acids or lipid depots. It was assumed that any residues of Rapeseed oil will be degraded or utilised by the plant by the time of harvest, so that it is indistinguishable from plant endogenous lipids and therefore residues trials were not reported. Nevertheless the metabolism takes place in plant tissues and inside the cell and it was not reported the ability of triacylglycerols to penetrate into the plant cells when they are applied on the plant. RMS considers that the reported literature is not conclusive about the behaviour of rapeseed oil when is applied <u>on the plant</u> and aditional data should be submitted before a conclusion about the residue definition is set. Although fatty acids occur naturally in plants, additional data/information are needed to confirm that "not naturally occurrence fatty acids" or undesirable compounds are not found in the plant as a consequence of the application of rapeseed oil.

The notifier did not provide any specific study on the metabolism of rapeseed oil in livestock but literature about the metabolism of fats was submitted. The fatty acids comprised in Rapeseed oil occur naturally in animal feed and their metabolism in animals is well known. Furthermore, Rapeseed oil is also used as a food commodity and therefore metabolism studies on rapeseed oil itself are not needed. Depending on the residue definition for plant material and the clarification on the intended uses, more information may be needed

Rapeseed oil is a food commodity which is consumed at about 7.3 g/day in the European diet. The extent of the exposure due to the intended use as plant protection product in apples was compared to the exposure due to consumption of Rapeseed oil as a food commodity. A very worst case, assuming that all the active substance applied would be uptake by the fruit, was considered. The estimation of consumption according to the European diet was much lower than the normal daily consumption of this commodity. Furthermore, it is applied early in the growing season before the development of fruits in orchards and therefore this consumption will be even lower. Therefore no residue trials were reported and no residue definition and MRLs were set and studies on the effect of industrial processing and/or household preparation on the nature and level of residues were therefore considered not relevant. Nevertheless RMS will do a definitive assessment on the residue definition when more information is available about plant metabolism.

Up to now, no MRLs have been established for Rapeseed oil by EU or European Member State.

Rapeseed oil is degraded rather rapidly in the soil and the fatty acids comprised in Rapeseed oil also occur naturally in soil. Furthermore, the active substance is used in permanent crops and greenhouses so that it is not expected that succeeding crops will occur. Studies on the effect on succeeding crops were therefore also considered not relevant.

No pre-harvest interval, re-entry period for livestock or withholding period for animal feeding stuffs have to be proposed. A re-entry period for man to crops or a waiting period between application and handling treated products are not relevant due to the toxicological profile and the rapid degradation of Rapeseed oil. A waiting period between application and sowing or planting succeeding crops is not required due to the rapid degradation of the active substance in the soil.

No EU Import tolerances exist for Rapeseed oil.

Estimations of TMDI, NEDI or NESTI are not applicable, since no MRLs are proposed for Rapeseed oil. Nevertheless more information about the residue definition for plant material and clarification on the intended uses is needed before a conclusion can be achieved.

B.7.17 References relied on

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protect. claimed	Owner
IIA 6.2.1/01	Ting, I.P.	1982	Plant Physiology not applicable Addison-Wesley Publishing Company, London Report-no. not applicable GLP: no published: yes	**No**	--
IIA 6.2.1/02	Galliard, T.	1978	Lipolytik and lipoxygenase enzymes in plants and their action in wounded tissues not applicable In: Biochemistry of wounded plant tissues, Walter de Gruyter & Co., Berlin, New York, 155-197 Report-no. not applicable GLP: no published: yes	**No**	--
IIA 6.2.1/03	Hitchcock, C., Nichols, B.	1971	Plant lipid biochemistry not applicable Academic Press, London Report-no. not applicable GLP: no published: yes	**No**	--
IIA 6.2.1/04	Rivera, C.M., Penner, D.	1979	Effect of herbicides on plant cell membrane lipids. not applicable Residue Reviews Vol. 70: 45-76 Report-no. not applicable GLP: no published: yes	**No**	--
IIA 6.2.1/05	Tso, T.C., Chu, H., DeJong, D.W.	1975	Residue levels of fatty compounds and surfactants as suckering agents on tobacco. not applicable Beiträge zur Tabakforschung. Vol. 8: 241-245 Report-no. not applicable GLP: no published: yes	**No**	--
IIA 6.2.1/06	Tso, T.C., Chu, H.	1977	The fate of fatty compounds and surfactants used a sucker control agents on field tobacco not applicable Beiträge zur Tabakforschung. Vol. 9: 58-62. Report-no. not applicable GLP: no published: yes	**No**	--
IIA 6.2.1/07	Murphy, D.J., Stumpf, P.K.	1980	In vivo pathway of oleate and linoleate desaturation in developing cotyledons of Cucumis sativus L. seedlings. not applicable Plant Physiology Vol. 66: 666-671 Report-no. not applicable GLP: no published: yes	**No**	--

Annex point/ reference number	**Author(s)**	**Year**	**Title** **Source** (where different from company) **Company name,** **Report No.,** **GLP status** (where relevant) **published or not**	**Data protect. claimed**	**Owner**
IIA 6.2.2/01 IIA 6.2.3/01 IIA 6.2.4/01	Guthrie, H.A.	1975	Fats or lipids not applicable In: Introductory Nutrition, 3rd Ed., Times Mirrow/ Mosby College Pub., St. Louis Report-no. not applicable GLP: no published: yes	**No**	--
IIA 6.2.2/02 IIA 6.2.3/02 IIA 6.2.4/02	Sinclair, H.M.	1964	Essential fatty acids not applicable Medical chemistry. Vol. 2: Lipid Pharmacology: 237-268. Report-no. not applicable GLP: no published: yes	**No**	--
IIA 6.3.1/01	Watt, B.K. & Merill, A.L.	1963	Composition of foods - raw, processed and prepared not applicable U.S. Dep. of Agriculture Handbook No. 8, Washington, D.C., U.S. Report-no. not applicable GLP: no published: yes	**No**	--

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