UNIVERSITÀ DEGLI STUDI DI MILANO

SELEZIONE PUBBLICA, PER TITOLI ED ESAMI PER IL RECLUTAMENTO DI N. 3 UNITÀ DI TECNOLOGO DI SECONDO LIVELLO CON RAPPORTO DI LAVORO SUBORDINATO A TEMPO DETERMINATO, DELLA DURATA DI 18 MESI CIASCUNO, PRESSO L'UNIVERSITÀ DEGLI STUDI DI MILANO - DIPARTIMENTO DI SCIENZE BIOMEDICHE E CLINICHE "L. SACCO" - BANDITO CON DETERMINA N. 13222 DEL30/10/2020 E PUBBLICATO SUL SITO INTERNET D'ATENEO IL 30/10/2020 – CODICE 21232

TRACCE PROVA SCRITTA

La Commissione giudicatrice della selezione, nominata con determina n. 1718 del 21/12/2020 e così composta:

Prof. Clementi Emilio Giuseppe Ignazio - Presidente

Prof. Moretto Angelo - Componente

Dott.ssa Metruccio Francesca - Componente

Dott.ssa Gentile Anna Concetta - Segretario

comunica le tracce relative alla prova scritta:

TEMA n. 1
Vedi allegato

TEMA n. 2
Vedi allegato

TEMA n. 3
Vedi allegato

La Commissione

Prof. Clementi giuseppe Ignazio - Presidente ……………………………………………

Prof. Moretto Angelo - Componente ……………………………………………

Dott.ssa Metruccio Francesca - Componente ……………………………………………

Dott.ssa Gentile Anna Concetta - Segretario ……………………………………………

PROVA SCRITTA 1

DOMANDE A SCELTA MULTIPLA (UNA SOLA RISPOSTA ESATTA)

1. il Regolamento CE 1107/2009

1. Sostituisce la Direttiva 91/414/CEE
2. Modifica la Direttiva 91/414/CEE
3. Integra la Direttiva 91/414/CEE
4. Sostituisce i Principi Uniformi con un nuovo e diverso approccio
5. Sostituisce i Principi Uniformi e introduce i criteri di cut-off

2. La correlazione dose risposta:

1. Correla la dose con l'entità/gravità dell'effetto
2. È sempre lineare
3. La sua definizione dipende dal giudizio dell'esperto
4. Ha una distribuzione a campana
5. Correla con il logaritmo della dose

3. Il pericolo è definito come:

1. Il tipo e la gravità di effetto tossico che viene causato da una sostanza
2. La capacità intrinseca di una sostanza o miscela di causare effetti avversi nell'uomo o nell'ambiente in seguito ad esposizione
3. L'entità del danno che si attende dopo esposizione ad una data sostanza
4. La gravità del danno in rapporto all'entità dell'esposizione
5. Il rapporto fra caratteristiche chimiche e danno alla salute

4. Un residuo di fitofarmaco non-conforme al LMR in un campione di riso è la conseguenza di?

1. Contaminazioni
2. Assenza di pioggia
3. Falsi positivi
4. Un TMDI maggiore di 100%
5. Il non rispetto dell'intervallo di sicurezza

5. Il coefficiente di ripartizione (per es: ottanolo/acqua) è:

1. Stabilito per legge
2. Una misura empirica della solubilità lipidica
3. Dipende dal giudizio dell'esperto
4. Una misura precisa dell'assorbimento cutaneo
5. Una caratteristica di sostanze lipofile

6. Il LOAEL (Lowest Observable Adverse Effect Level)

1. È l'effetto tossico meno grave
2. È la dose più alta alla quale si osservano effetti avversi nell'uomo
3. È la dose più bassa alla quale si osservano effetti tossici
4. È la dose più alta alla quale non si osservano effetti avversi
5. È qualsiasi dose alla quale si osservano effetti tossici

7. Il bioaccumulo

1. È il processo attraverso cui sostanze organiche persistenti si accumulano all'interno di un organismo, in concentrazioni superiori a quelle riscontrate nell'ambiente circostante.
2. È un fenomeno di accumulo crescente di una sostanza lungo una catena trofica: nell'organismo predatore la concentrazione (normalizzata sul contenuto lipidico) risulta più alta rispetto a quella rilevabile nelle prede.
3. È un fenomeno di accumulo di sostanze nutritive da parte dei microorganismi del suolo
4. È il processo attraverso cui le piante assorbono nutrimenti organici con l'apparato radicale
5. È il fenomeno di accumulo nel suolo di una molecola organica caratterizzata da un'elevata persistenza

PROVA SCRITTA 1

Fate and behaviour in the environment Study

Data point:	**CA 7.1.1.1/01, Doe, R. A. (1994)**
Report author	Doe, R. A
Report year	1994
Report title	Aerobic soil metabolism of [^{14}C]XXX
Report No	RPT00204
Document No:	NA
Guidelines followed in study	US EPA Subdivision N, § 162-1 Deviations: None

Executive Summary
An aerobic laboratory soil degradation study was conducted with [^{14}C] XXX in North Dakota sandy loam soil under aerobic conditions at an application rate of *ca*. 1 mg/kg. Soil was incubated in the dark at a temperature of 25.0 °C ± 1.0 °C and a soil moisture of 75% of 33kPa for up to 181 days.

XXX degraded at an extremely rapid rate during the course of the study. A very polar fraction designated as Metabolite A reached a maximum of 5.8 % after 58 days incubation. No other significant metabolites (>5 %) were detected.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material** [^{14}C] XXX
   Lot/Batch: CFQ7390 (B# NB 8309)
   Specific activity: 425.5 mBq/mmoL
   Purity: Radiochemical purity >96 %

2. **Soil:**

The soil characteristics are summarised in

Table Errore. Nel documento non esiste testo dello stile specificato.**-1**.

Table Errore. Nel documento non esiste testo dello stile specificato.**-1: Chemical and Physical Characteristics of Test Soil**

Soil name	North Dakota
Site location	USA
pH	6.4
Organic carbon [%]	2.2
% Organic mater	3.7
[a]CEC (meq/100g)	18.0
Bulk density (g/cc)	1.08
Soil type (according to USDA)	Sandy loam
Particle size analyses according to USDA	
Clay:	14
Silt:	18
Sand:	68
Moisture at 33kPa (%)	20.4

[a] Cation exchange capacity

B. STUDY DESIGN AND METHODS

1. Experimental Conditions

An aerobic laboratory soil degradation study was conducted with [^{14}C] XXX in North Dakota sandy loam soil. Portions of sieved and mixed soil (50 g, < 2 mm) were adjusted to *ca*. 75 % of 33 kPa moisture capacity and treated with the active substance dissolved in acetonitrile. Thirty samples were treated at *ca*. 1 mg/kg treatment rate. Each soil sample weighed 50 g on a dry weight basis and was treated with 60 µL of dosing solution.
Four samples were treated at a high dose application rate of *ca*. 10 mg/kg by the addition of 70 µL of the dosing solution. Two sets of control samples were prepared in a similar manner; however, they were fortified with just acetonitrile. Six additional flasks were prepared at *ca*. 10 mg/kg rate for metabolite product identification. The flasks were incubated under aerobic conditions in the dark at 25.0 °C ± 1.0 °C for up to 272 days.
The samples were thoroughly mixed, and a polyurethane plug was inserted between the soil and the sidearm to trap neutral volatiles (10mL of 1N KOH). This trapping method was not employed in the definitive phase of the study since it was deemed unnecessary, based on the results of the preliminary work.

2. Sampling

Samplings of the *ca*. 1 mg/kg dosed samples were performed on days 0, 0.5, 1, 2, 3, 7, 14, 30, 59, 121, and 181 post treatment. At each sampling point, duplicate flasks were harvested for analysis. The *ca*. 10 mg/kg dosed samples were harvested at days 14 and 121 after treatment. $^{14}CO_2$ and volatiles were collected *ca.* biweekly or at each sampling time point from the test flasks via a KOH trapping system and duplicate aliquots were analysed by LSC. Trapped radioactivity was confirmed to be $^{14}CO_2$ by barium salt precipitation of aliquots from days 14 and 226, followed by LSC analysis.

3. Description of analytical procedures

All soil specimens were extracted with acidified acetonitrile before being vacuum filtered yielding a filtrate and a bound residue soil fraction. The filtrate was partitioned twice with dichloromethane

yielding an organic and an aqueous fraction which were quantified by LSC. The residual soil fraction was air dried and aliquots were combusted and assayed by LSC.
Bound residue analysis was performed on soil samples by acid reflux (0.25 N HCl solution), humic and fulvic acid determinations followed by LSC. Reverse phase HPLC, TLC and gas chromatography were performed to identify degradation products.

II. RESULTS AND DISCUSSION

A. DATA

XXX was shown to be degraded extremely rapidly. Table Errore. Nel documento non esiste testo dello stile specificato.**-2** below shows the distribution of radioactivity following application. This pattern suggests that the extractable organics, including parent XXX, metabolise to bound residues, which thereafter metabolise to CO_2.
The analysed composition of extractable organics is presented below in Table Errore. Nel documento non esiste testo dello stile specificato.**-2**. XXX accounted for a majority of the soil organo-extractable product profiles through the initial 7-day period of incubation. Total XXX in the organic extracts declined from an average level of 85.42 % of the AR on day 0 to less than 1 % by day 59 of the study.

Table Errore. Nel documento non esiste testo dello stile specificato.**-2: Average percent distribution of radioactivity in soil organosoluble fractions at various intervals**

Fraction ID	[a]CH_3CN/H_2O (% total)	[b]CH_3CN/CH_2Cl_2 (% total)	[c]Aqueous-1 (% total)	[d]PES (% total)	[e]KOH (% total)	Total recovery (% total)
1 mg/kg						
Day 0	91.2	91.1	0.07	2.4	n.a	93.6
Day 0.5	81.9	81.6	0.28	11.4	0.32	93.6
Day 1	83.8	83.4	0.42	12.3	1.03	97.1
Day 2	71.7	71.0	0.70	17.2	3.23	92.1
Day 3	61.1	59.9	1.19	23.5	4.99	89.6
Day 7	39.9	37.4	2.52	36.9	12.3	89.1
Day14	30.0	27.5	2.44	40.0	22.4	92.4
Day 30	20.9	19.0	1.91	40.0	31.8	92.7
Day 59	15.0	13.0	2.01	38.6	37.7	91.3
Day 121	8.5	7.1	1.31	35.1	41.4	84.9
Day 181	9.1	7.4	1.70	34.0	43.4	86.5
Average Total Recovery						91.2
10 mg/kg						
Day14	60.1	57.1	3.12	25.2	3.13	88.5
Day 121	10.8	9.94	0.86	33.5	n.a	44.3

[a] Total recovery, as filtrate, yielded from extraction from biometer flasks. This filtrate was fractioned into an organic (b) and an aqueous (c) fraction
[b] Organosoluble acetonitrile/methylene chloride fraction (organic fraction), including XXX and metabolites
[c] Aqueous fraction
[d] Post extraction solids (equivalent to soil bound residues)
[e] Equivalent to carbon dioxide and volatiles
n.a. Not applicable
PES Post-extraction solid

Table Errore. Nel documento non esiste testo dello stile specificato.**-3: Average percent distribution of XXX and metabolites in soil organosoluble fractions (CH_3CN/CH_2Cl_2) at various intervals (1 mg/kg)**

Day	% of total organosoluble fractions (CH_3CN/CH_2Cl_2)									% of total recovery
	[^{14}C]XXX	Met-A	Met-B	Reg-1	Met-C	Reg-2	Met x	Reg-3	Reg-4	
0	85.4	n.d	n.d	n.d	n.d	n.d	1.11	4.59	n.d	91.12
0.5	65.3	n.d	n.d	n.d	n.d	n.d	2.67	10.90	2.71	81.62
1	65.4	n.d	n.d	n.d	n.d	n.d	2.43	12.49	3.04	83.35
2	48.2	n.d	1.24	n.d	1.10	2.38	2.48	13.21	2.39	71.01
3	15.6	n.d	2.03	n.d	1.79	3.90	4.01	29.73	2.85	59.88
7	11.9	1.25	3.63	n.d	2.28	2.96	1.78	11.08	2.52	37.37
14	4.02	4.72	2.33	1.56	n.d	3.20	0.87	8.10	2.73	27.51
30	2.08	3.71	2.14	n.d	0.44	3.28	0.40	5.97	0.99	18.99
59	0.52	5.82	1.62	0.48	n.d	1.33	0.27	2.99	n.d	13.00

n.d not detected
Met metabolite
Reg regions

The "region 3" (as used in chromatograms) is a very wide area comprising the retention times from 23 min until 35 min (considering a total elution time of 50 minutes).
From day 0 until day 2 (13.21% AR) only minor peaks occur in this region. On day 3 (29.73% AR) this region is formed by 3 main peaks and a numerous number of minor peaks. Although those 3 peaks have not been quantified, a visual comparison with XXX peak can be done in order to have an estimation of their representativeness: These 3 peaks are of considerably minor area than XXX – which on day 3 represents only 15.58%. Hence, it is concluded that they individually represent an area corresponding to significantly less than 10% AR. On top of that, on day 7, the region 3 (11.08% AR) is formed again by numerous negligible peaks. Hence, the 3 peaks occurring on day 3 are of transient behaviour. For these reasons, the region 3 is considered to be not relevant for the metabolism of XXX in soil.
No attempts were made to quantify residues of XXX and its metabolites in the day 120 and day 180 organic extracts samples. This was due to the abundance of components present in these samples and the apparent low contribution of each to the total radioactivity applied to the system.
Analysis of volatile traps indicated that the evolved acidic volatiles trapped in KOH solution formed rapidly from day 1 (average of 1%) to the end of the definitive phase of the study (an

average of 43.41% by day 181). The rate of volatile formation was also rapid, reaching a plateau by day 59 of the study.

Analysis of 0.5 mL aliquots of day 14 and day 226 KOH samples by barium chloride precipitation showed that greater than 90% of the radioactivity in the samples could be precipitated as [^{14}C]barium carbonate, thus verifying that a majority of the trapped volatiles was attributable to $^{14}CO_2$.

Fractionation of the post-extraction solids showed that about 10 %, 12 %, and 6 % of AR was present in humic acid, fulvic acid, and humin fractions, respectively. HPLC and TLC analysis of an extract of the fulvic acid fraction showed the presence of the met x (reference compound).

B. MASS BALANCE

The mean mass balance over the entire incubation was 91.16 % for the low (1 mg/kg) treatment level.

C. BOUND RESIDUES

During the course of the study there was an increase in bound residue (PES) from an average of 2.40 % on day 0 to a maximum of 40.0 % by day 30, then a decrease to 34.0 % by day 181 of the study. The bound residue formation was very rapid; reaching an apparent steady state by day 14 of the study.

D. VOLATILISATION

Analysis of volatile traps indicated that the evolved acidic volatiles trapped in KOH solution formed rapidly from day 1 (average of 1 %) to the end of the definitive phase of the study (an average of 43.41 % by day 181). The rate of volatile formation was also rapid, reaching a plateau by day 59 of the study.

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E. METABOLITES

Polar metabolites, designated as metabolites A and B were observed from day 7 to the end of the study. The products eluted very early and may be comprised of more than one component, but further resolution of metabolites was not attainable due to their polarity. Metabolites C and D were less polar compared to metabolites A and B, but chromatographed as relatively broad peaks, suggesting they may be comprised of more than one component. Metabolite C appeared by day 2 of the study (1.10 %), peaked at 2.28 % by day 7, and declined to near or at the detection limit by day 14 of incubation. Metabolite D, which exhibited a retention time very similar to met x reference chemical, appeared very early in the study (day 0), amounted to a maximum of ~4 % of the AR by day 3, and then declined to less than 1 % by day 14 of incubation. Four non-specific regions of radioactivity were quantified (Reg 1-4) and these contained multiple components that could not be resolved.

III. CONCLUSIONS

XXX degrades at a very rapid rate when applied to soil and incubated under aerobic conditions. Degradation proceeds initially by a combination of hydrolysis and oxidation to form a number of low-level metabolites. Residues in soil are initially extracted, but extended degradation is accompanied by the formation of residues that are bound to sediment humus fractions. Soil residues are ultimately bound to soil humus fractions and mineralised (converted to carbon dioxide). No individual metabolite exceeded 5% at successive timepoints. It can further be inferred from the mass balance data that Met-A had reached a maximum value after 59 days

Comments:

PROVA SCRITTA 1

Ecotoxicology Study

Report:	**Doe . 2009a**
Title:	**Acute oral toxicity (LD_{50}) study of XXX in Japanese quail**
Report No.:	**00001**
Guidelines:	**SETAC N° 1.1.1 Assessing the Environmental Fate and Ecotoxicity of Pesticides, Part 2 Ecotoxicity, N° 1. Vertebrate Terrestrial Wildlife, N° 1.1 Birds, N° 1.1.1 "Acute Toxicity", March 1995**
Deviations:	**None**
GLP:	**Yes**

Materials and Methods

MATERIALS:

Test Material: XXX
Description: Solid chunks/dark brown
Lot/Batch #: 1364474
Purity (according to CoA): 99.75%
CAS #: 9576-1
Stability of test compound: Expiry date: October 05, 2009
Vehicle and/or positive control: Corn oil was used as vehicle for XXX
Test animals
Species: *Coturnix japonica*
Age: Adult, more than 12 weeks old
Weight at dosing: Each bird was weighed prior to administration of the dose.
Source: Commercial supplier
Acclimation period: 7 days
Diet: Japanese quail layer mash diet (Amrut brand, manufactured by Pranav Agro Industries Ltd., Pune, Maharashtra, India)
Water: Water was provided *ad libitum*
Housing: Birds were housed individually in a stainless steel wire mesh bottomed pen with floor area of 900 cm^2.
Loading: Range finding test: 2 males and 2 females
Main study: 3 males and 3 females
Environmental conditions:
Temperature: 21 - 24° C

Humidity: 59 – 76 %
Air changes: Well-ventilated
Photoperiod: 8 h light and 16 h darkness

STUDY DESIGN AND METHODS:

In life dates: Experimental Starting Date: 20 March 2009
Experimental Completion Date: 03 April 2009

Animal assignment and treatment:

Each bird was identified by a leg band.

XXX was weighed and made suspension with corn oil to make a gavage mixture for the treated group. All birds were administered a single oral dose using 1.0 mL glass syringe. The volume administered to each bird was calculated according to its body weight. Birds from the control group were dosed with corn oil. The dose was 10.0 mL/kg body weight for all the groups. Dose levels, were 80, 160 and 320 mg XXX/kg body weight. After dosing, the birds were placed back in their cages.

Observations:

All the birds were observed for signs of toxicity and mortality at 30 minutes, 1 and 2 hours post dosing on day 0 and subsequent once daily for 14 days. Body weight was recorded individually prior to dosing on day 0 and at the termination of the experiment on day 14. Cage wise feed consumption was recorded daily and calculated for period 0 – 3, 4 – 7 and 8 – 14. All the animals which dies during the study and those humanely sacrificed by carbon dioxide asphyxiation at the end of the observation period were subject to pathological examination.

Statistics:

The LD_{50} value was calculated from the observed mortality data, using the Probit analysis method (Finney, 1971). The bodyweight and feed consumption data were subjected to statistical analysis by using student's "t" and ANOVA test.

RESULTS AND DISCUSSION

MORTALITY AND SYMPTOMS OF TOXICITY

The mortalities observed were 33.3, 83.3 and 100% at the dose level of 80, 160 and 320 mg XXX/kg body weight. No mortality was observed in the control group (see the following table).

Table CA9. 1: Mortality data

Dose (mg XXX/kg b.w.)	Concentration mg/mL	Doses volume mL/kg b.w.	No of birds/group (3M + 3F)	Mortality	
				Up to 14 days	%
0 control	-	10	6	0	0
80	8.0	10	6	2	33.3
160	16.0	10	6	5	83.3
320	32.0	10	6	6	100

b.w. = body weifht, M = Male, F = Female

CLINICAL OBSERVATION

Birds in the control group did not exhibit abnormal behaviour symptoms from the day of dosing until the end of the observation period (14 day). The toxicity symptoms observed in 80, 160 and 320 mg/kg body weight were moribund, hyperactive, gasping and lethargy.

BODY WEIGHT

Individual body weight for male and female birds was recorded on day 0 and 14 and the data were subjected to statistical analysis. Statistically no significant differences were observed in the body weight on day 0 from all the treatment group birds when compared with the control group. Due to mortality in mid dose and high dose group statistical analysis was not possible on day 14 (for more details see the following table).

Table CA9. 2: Group wise mean body weight (g)

Group	Dose (mg XXX/kg b.w.)	Number of birds			Mean body weight (g) on day			
					0		14	
		M	F	T	M	F	M	F
G1	0 (control)	3	3	6	172.00 ± 20.52	185.67 ± 26.63	191.33 ± 22.12	196.33 ± 22.59
G2	80	3	3	6	169.00 ± 15.72	185.00 ± 26.63	182.50 ± 4.95	200.00 ± 12.73
G3	160	3	3	6	174.67 ± 5.51	187.67 ± 11.59	176.0	-
G4	320	3	3	6	171.00 ± 6.00	181.67 ± 14.57	-	-

FEED CONSUMPTION

Individual bird feed consumption (male and female) were recorded daily and the data was subjected to statistical analysis. Statistically no significant differences were observed in the feed consumption on post-dosing 0 – 3, 4 – 7 and 8 – 14 days in male and female birds from all the treatment groups when compared with the control group, except male birds from low dose group statistically significant different observed on day 4 – 7 when compared with the control group. Due to mortality in mid and high dose group statistical analysis was not possible (for more details see the following table).

Table CA9. 3: Group wise mean feed consumption (g/bird)

Group	Dose (mg XXX/kg b.w.)	Number of birds			Feed consumption (g/bird) on day					
					0-3		4-7		8-14	
		M	F	T	M	F	M	F	M	F
G1	0 (control)	3	3	6	67.33 ± 37.69	320.67 ± 40.55	138.33 ± 19.35	285.67 ± 45.35	335.33 ± 94.73	690.00 ± 39.28
G2	80	3	3	6	47.67 ± 41.96	130.33 ± 115.09	300.00 ± 67.88	326.00 ± 79.20	258.00 ± 83.44	638.50 ± 119.50
G3	160	3	3	6	29.67 ± 51.38	-	317.00	-	461.00	-
G4	320	3	3	6	-	-	-	-	-	-

NECROPSY EXTERNAL AND INTERNAL

External: all surviving birds were subjected to gross pathological examinations at the end of the 14 day observation period. External examination of the found dead and terminally sacrificed birds did not reveal any lesion of pathological abnormality.

Internal: visceral examination of terminally sacrificed birds from the control and different treatment groups did not reveal any lesion of pathological significance. However, visceral examination of found dead birds from treatment groups revealed varying degree of lesions like lung : congestion (Bird No. 9, 13, 15, 18, 19, 20, 22, 23 and 24); kidney : congestion (Bird No. 9, 10, 13, 17, 18 and 23) and brain : congestion (Bird No. 15 and 20).
It was found that birds from low (1/6), mid (5/6) and high (6/6) shows mortality. On the basis of lesions observed in the found dead birds belonging to the treatment groups, it could be concluded that the above recorded lesions might be correlated with the test substance. For more details see the following tables.

Table CA9. 4: Necropsy findings of individual bird – Group G1: (Control) 0 mg/kg b.w.

Bird No./Sex	Mode of death	Gross findings	
		External	Internal
1 M	TS	NAD	NAD
2 M	TS	NAD	NAD
3 M	TS	NAD	NAD
4 F	TS	NAD	NAD
5 F	TS	NAD	NAD
6 F	TS	NAD	NAD

Table CA9. 5: Necropsy findings of individual bird – Group G2: 80 mg/kg b.w.

Bird No./Sex	Mode of death	Gross findings	
		External	Internal
7 M	TS	NAD	NAD
8 M	TS	NAD	NAD
9 M	FD	NAD	Lungs: Congestion (+) Kidney: Congestion (+)
10 F	FD	NAD	Kidney: Congestion (1+)
11 F	TS	NAD	NAD
12 F	TS	NAD	NAD

Table CA9. 6: Necropsy findings of individual bird – Group G3: 160 mg/kg b.w.

Bird No./Sex	Mode of death	Gross findings	
		External	Internal
13 M	FD	NAD	Lungs: Congestion (+) Kidney: Congestion (2+)
14 M	TS	NAD	NAD
15 M	FD	NAD	Lungs: Congestion (+) Kidney: Congestion (+)
16 F	FD	NAD	NAD
17 F	FD	NAD	Kidney: Congestion (+)
18 F	FD	NAD	Lungs: Congestion (+) Kidney: Congestion (+)

Table CA9. 7: Necropsy findings of individual bird – Group G4: 320 mg/kg b.w.

Bird No./Sex	Mode of death	Gross findings	
		External	Internal
19 M	FD	NAD	Lungs: Congestion (1+)
20 M	TS	NAD	Lungs: Congestion (1+) Brain: Congestion (+)
21 M	FD	NAD	NAD
22 F	FD	NAD	Lungs: Congestion (+)
23 F	FD	NAD	Lungs: Congestion (1+) Kidney: Congestion (+)
24 F	FD	NAD	Lungs: Congestion (+)

CONCLUSIONS

The acute oral toxicity (LD_{50}) of XXX in Japanese quail (*Coturnix japonica*) was found to be 99.09 mg XXX/kg body weight. The 95% fiducial limits to LD_{50} were found to be between 66.42 and 147.83 XXX/kg body weight.

Comments:

PROVA SCRITTA 1

Mammalian toxicology Study

Compound XXX (purity 98.4%) was administered to four groups 10 animals/sex/group of approximately 7-week-old Han Wistar rats (Crl:WI(Han)) at a dietary concentration of 0, 100, 500, 1500 (females) or 2500 ppm (males) (equal to 0, 6.2, 31.5 and 159 mg/kg bw per day in males and 0, 7.0, 36.1 and 110 mg/kg bw per day in females, respectively) for 90 days. All animals were generally checked twice each day for viability. Once each week starting pre-trial, all animals received a detailed clinical examination (appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta). Body weight was measured twice weekly during pre-trial and weekly during the treatment period. Food consumption was measured once a week during pre-trial and treatment period. Visual inspection of water consumption was performed throughout the study. Ophthalmoscopic examination was conducted once during the pre-trial period and all control and high dose animals were examined during week 13 of treatment. All animals received a detailed functional observation battery assessment (including motor activity) during week 12 of treatment. An adequate number of haematological, coagulation, clinical chemistry and urine parameters were evaluated. All surviving animals were euthanised after 90 days of treatment and subjected to a detailed necropsy examination. The following organs were weighed (brain, epididymides, adrenals, pituitary, prostate, heart, kidneys, liver, thymus, ovaries/testes, spleen and uterus/cervix, thyroid with parathyroid). An adequate number of tissues was sampled for microscopic examination. Histopathology was conducted all control and high dose group animal on all tissues. Additionally, all gross lesions, the thyroid gland from both sexes and the harderian gland from females only were processed, from low and intermediate dose animals.

No mortality and clinical sing of toxicity were reported.

Statistically significant decreases in body weight gain (up to 20%) and body weight (up to 9%) were noted in males at 2500 ppm and females at 1500 ppm. In males at 500 ppm, decrease of body weight was reported (approximately of 8% from control), achieving statistical significance from day 7 to 77 in males. In females at 500 ppm. Statistically significant lower body weight gains were noted in both sexes, on interval days 0-28, 0-70, and 0-77. Statistically significant food consumption decrease was noted in males at 2500 ppm and females at 1500 ppm over the first 4 weeks of treatment. Animals at 100 ppm and 500 ppm showed food consumption values similar to controls except for week 1 and 2 of treatment. Food utilization was significantly lowered in the first 4 weeks for both sexes at the high dose, which in females resulted in a statistically significant food utilization decrease the for the overall exposure period.

Statistically significant decrease of foot splay was reported in males at 2500 ppm and females at 1500 ppm when compared to controls. Males at 2500 ppm showed a statistically significant decrease of hind grip.

White blood cell count, neutrophils, lymphocytes and basophils were significantly higher in males at 2500 ppm when compared with controls. Statistically significant increase of prothrombin time was reported in males at 2500 ppm.

A statistically significant increase of ALT was reported in males at 2500 ppm and in females at 500 ppm and 1500 ppm. AST was statistically significant increased in males at 2500 ppm. A dose related decrease of cholesterol was reported in both sexes, achieving statistical significance at the mid and high dose of approximately 30% and 50%, respectively, in both sexes. A statistically significant and dose related decrease of triglycerides of 60% was reported in in males at 2500 ppm, and of approximately 50% in females at 500 ppm and 1500 ppm. Total proteins and albumin decrease in a statistically significant manner in males at 500 ppm and 2500 ppm and in females at 1500 ppm. A statistically significant decrease of calcium was reported in females at 1500 ppm.

A statistically significant lower absolute and covariate adrenal gland weights of approximately 15% was recorded in males at 500 or 2500 ppm. A statistically significant lower absolute and covariate spleen weight were reported in females at 500 or 1500 ppm of approximately 15%, when compared with controls. Absolute kidney weight was statistically significantly lower in males at 2500 ppm when compared with controls.

No gross findings were noted.

Microscopic findings consisted of minimal to mild diffuse follicular cell hypertrophy and colloid contraction in the thyroid glands of males at 2500 ppm and females at 1500 ppm.

Key findings of the 90 day dietary toxicity study in rat

Parameters		Males				Females			
		0	100 ppm	500 ppm	2500ppm	0	100 ppm	500 ppm	1500 ppm
Body weight (g)									
	Day								
	0	224	213	215	220	153	157	154	151
	3	234	222	224	215**	159	163	160	154
	7	252	240	239*	232**	169	174	169	162
	14	282	267	267*	255**	187	186	184	174
	28	326	314	307*	291**	213	214	204	195*
	56	370	355	347*	332**	229	230	219	211
	91	400	385	377	364**	240	240	230	221
Body weight gain (g)									
	Day								
	0-3	10	9	9	-5**	7	6	6	3

Parameters	Males				Females			
	0	100 ppm	500 ppm	2500ppm	0	100 ppm	500 ppm	1500 ppm
0-7	28	27	24	12**	17	17	15	12
0-14	59	54	52	35**	34	29	30	24**
0-28	102	101	92	71**	60	56	51*	45**
0-56	146	141	132	112**	76	73	66	61**
0-77	171	165	151*	134*	85	80	73*	68**
0-91	176	172	162	144**	88	82	77	70*
Food consumption (g/animal/day)								
Week								
1	22.9	20.7**	21.3*	18.3**	15.7	15.3	14.8	13.2**
2	24.5	21.5**	23.3	21.1**	17.3	15.9	16.9	16.0
4	22.3	21.7	20.8	19.8**	16.5	16.1	15.6	15.2
7	19.3	17.9	17.9	18.1	14.4	14.7	14.2	13.6
13	18.9	18.2	18.6	19.1	13.5	13.6	13.6	13.0
Mean 1-4	23.4	21.6	22.0	20.1	16.7	16.3	16.2	15.2
Mean 1-13	20.6	19.4	19.5	19.0	15.2	14.9	14.8	14.4
Food utilization (g body weight gain/g food consumed)								
Week								
1–4	15.6	16.7	14.9	12.6**	12.9	12.4	11.2	10.4**
1–13	9.4	9.7	9.1	8.3	6.3	6.1	5.7	5.4*
Functional observation								
Foot splay (mm)								
Pre-trial	80	80	77	76	78	76	78	79
Week 12	99	103	90	72*	96	88	82	64*
Hind grip (g)								
Pre-trial	262	229	230	289	242	260	262	265
Week 12	529	485	478	438*	434	411	402	347

Parameters	Males				Females			
	0	100 ppm	500 ppm	2500ppm	0	100 ppm	500 ppm	1500 ppm
Haematology								
WBC	6.42	6.34	6.86	9.19**	4.50	5.13	5.35	5.27
neutrophils	0.98	1.16	1.02	1.64**	0.57	0.63	0.73	0.65
lymphocytes	5.12	4.85	5.55	7.16**	3.64	4.22	4.35	4.39
basophils	0.02	0.02	0.02	0.04*	0.02	0.02	0.01	0.02
PT	24.1	25.2	26.7	26.9*	25.2	25.3	25.2	24.9
Clinical biochemistry								
ALT (U/L)	43	41	41	78**	37	37	49*	63**
AST (U/L)	63	64	63	100*	61	58	73	63
Cholesterol (mmol/L)	1.8	1.5	1.2**	1.0**	1.5	1.4	1.0**	0.8**
Triglycerides (mmol/L)	1.69	1.35	1.46	0.67**	1.45	1.31	0.78**	0.69**
Total protein (g/L)	61	60	58*	57**	65	66	64	61**
Albumin (g/L)	41	41	39**	38**	48	47	46	44**
Calcium (mmol/L)	2.65	2.62	2.64	2.69	2.70	2.70	2.69	2.62*
Organ weight								
Adrenals								
Absolute (g)	0.0553	0.0507	0.0462**	0.0455**	0.0707	0.0684	0.0656	0.0620
Covariance with BW	0.0547	0.0507	0.0462*	0.0460*	0.0697	0.0676	0.0660	0.0636
Relative body weight (%)	0.01398	0.01349	0.01236	0.01266	0.02990	0.02897	0.02899	0.02822
Spleen								
Absolute (g)	0.60	0.55	0.54	0.56	0.48	0.44	0.41*	0.39**
Covariance with BW	0.58	0.55	0.54	0.58	0.47	0.43	0.41*	0.40*
Relative body weight (%)	0.152	0.145	0.144	0.155	0.204	0.188	0.180	0.179
Kidney								
Absolute (g)	2.15	2.05	2.03	1.94*	1.48	1.46	1.44	1.35*

Parameters	Males				Females			
	0	100 ppm	500 ppm	2500ppm	0	100 ppm	500 ppm	1500 ppm
Covariance with BW	2.07	2.04	2.04	2.02	1.45	1.43	1.44	1.39
Relative body weight (%)	0.544	0.540	0.541	0.539	0.627	0.617	0.630	0.620
Histopathology								
Thyroid								
No. examined	10	0	10	10	10	0	10	10
Follicular cell hypertrophy, diffuse								
total	0	NE	0	4	0	NE	0	7**
minimal	0	NE	0	1	0	NE	0	4
mild	0	NE	0	3	0	NE	0	3
Contraction, colloid								
total	0	NE	0	8***	0	NE	0	4
minimal	0	NE	0	3	0	NE	0	1
mild	0	NE	0	5*	0	NE	0	3

*: $P < .05$ **: $P < 0.01$; (analysis of variance, dose–response and Dunnett's); NE: not examined;

Conclusion:

PROVA SCRITTA 1

Methods of Analysis Study

Title:	Determination of ACTIVE SUBSTANCE 1 and ACTIVE SUBSTANCE 2 in formulations - Assay HPLC, external standard
Guidelines:	EU Directive 91/414/EEC, Annex III 5.1
GLP	Non GLP

The HPLC method AM014110MF1 is applicable for the determination of the content of ACTIVE SUBSTANCE 1 and ACTIVE SUBSTANCE 2 in formulations (20 + 100 g/L)).

Principle of the method:
Sample are dissolved in acetonitrile and placed in an ultrasonic bath for 15min. The flask is filled up with water and mix. The components are separated from formulation components and from each other on a reversed phase column (Zorbax Extend C18 or equivalent quality) using gradient elution ((1 L water + 5 mL 1 N sulphuric acid) // (Acetonitrile / tetrahydrofuran / methanol 10/5/5 (v/v/v)) = 62 // 38 for separation of ACTIVE SUBSTANCE 1 followed by 50 // 50 for separation of ACTIVE SUBSTANCE 2. The active ingredient ACTIVE SUBSTANCE 2 is stabilized by L-cysteine-hydrochloride-monohydrate. After UV detection (210 nm for A.S. 1 and 254 nm for A.S. 2), the quantitative evaluation is carried out by comparing the peak areas with those of reference substances, using an external standard.

Validation

Title:	Validation of HPLC-method AM014110MF1 - Determination of ACTIVE SUBSTANCE 1 and ACTIVE SUBSTANCE 2 in formulations - ACTIVE SUBSTANCE 1 + ACTIVE SUBSTANCE 2 FS 120 (20+100 g/L)
Document No:	M-367104-01-1 VB1-AM014110MF1
Guidelines:	EU Directive 91/414/EEC, Annex III 5.1 SANCO/3030/99 rev. 5
GLP	yes

Test item: ACTIVE SUBSTANCE 1 + ACTIVE SUBSTANCE 2 FS 120 (20+100g/L), batch 2009-010331
Blank matrix batch 2010-002009
Reference item

- ACTIVE SUBSTANCE 1, batch 81229,purity 98.7% w/w
- ACTIVE SUBSTANCE 2: batch SEEB80-811, purity 99.8% w/w

Retention time:

- ACTIVE SUBSTANCE 1 0.85min
- ACTIVE SUBSTANCE 2 3.35min

<table>
<tr><td>Specificity</td><td colspan="2">The UV-spectra of analytes in the sample and reference items show no spectral difference; the retention times of the analytes and reference items are identical</td></tr>
<tr><td>Interference</td><td colspan="2">Chromatograms were provided for reference items, test item, blank formulation. No interferences were found at the retention time of ACTIVE SUBSTANCE 2 and ACTIVE SUBSTANCE 1. Specificity is acceptable.</td></tr>
<tr><td rowspan="4">Linearity</td><td colspan="2">6 concentrations (single injections), range: 50 - 150 % of expected concentration. The functions are linear in the operation range. Linearity is acceptable.</td></tr>
<tr><td>Compound</td><td>Linearity</td></tr>
<tr><td>ACTIVE SUBSTANCE 1</td><td>Range concentration : 0.0474 to 0.151mg/mL
Correlation coefficient r: 0.97
Regression equation: y = 54.085749x -1.830210</td></tr>
<tr><td>ACTIVE SUBSTANCE 2</td><td>Range concentration : 0.243 to 0.825mg/mL
Correlation coefficient r: 0.99
Regression equation: y = 33.227584x +1.614318</td></tr>
<tr><td rowspan="4">Precision</td><td colspan="2">6 samples (single injection) from one batch (test item); assessment of repeatability.</td></tr>
<tr><td>Compound</td><td>Precision</td></tr>
<tr><td>ACTIVE SUBSTANCE 1</td><td>Relative standard deviation (RSD): 2.3 %
RSDr (with C=1.91%): 2.43%</td></tr>
<tr><td>ACTIVE SUBSTANCE 2</td><td>Relative standard deviation (RSD): 1.17 %
RSDr (with C=9.4%): 1.91%</td></tr>
<tr><td rowspan="2">Accuracy</td><td colspan="2">5 or 6 samples of laboratory-prepared synthetic formulation.</td></tr>
<tr><td>Compound</td><td>Accuracy</td></tr>
</table>

<table>
<tr><td></td><td>ACTIVE SUBSTANCE 1</td><td>Blank formulation fortified at 1.05-1.51-2.10-2.56-2.89% with technical ACTIVE SUBSTANCE 1

<table>
<tr><th>ACTIVE SUBSTANCE 1 added (%w/w)</th><th>ACTIVE SUBSTANCE 1 determined (%w/w)</th><th>Recovery %</th></tr>
<tr><td>1.05</td><td>1.08</td><td>94.2</td></tr>
<tr><td>1.51</td><td>1.55</td><td>105.4</td></tr>
<tr><td>2.10</td><td>2.13</td><td>103.4</td></tr>
<tr><td>2.56</td><td>2.60</td><td>101.6</td></tr>
<tr><td>2.89</td><td>2.91</td><td>100.7</td></tr>
</table>

Mean recovery: 101.1 %
Confidence interval of recovery: 101.1 ± 4.2</td></tr>
<tr><td></td><td>ACTIVE SUBSTANCE 2</td><td>Blank formulation fortified at 4.76-6.86-9.09-9.59-11.60-15.50% with technical ACTIVE SUBSTANCE 2

<table>
<tr><th>ACTIVE SUBSTANCE 2 added (%w/w)</th><th>ACTIVE SUBSTANCE 2 determined (%w/w)</th><th>Recovery %</th></tr>
<tr><td>4.76</td><td>4.79</td><td>100.6</td></tr>
<tr><td>6.86</td><td>6.91</td><td>100.7</td></tr>
<tr><td>9.09</td><td>9.10</td><td>100.1</td></tr>
<tr><td>9.59</td><td>9.64</td><td>100.5</td></tr>
<tr><td>11.60</td><td>11.60</td><td>100.0</td></tr>
<tr><td>15.50</td><td>15.50</td><td>100.0</td></tr>
</table>
Mean recovery: 100.3 %
Confidence interval of recovery: 100.33 ± 0.35</td></tr>
</table>

Conclusion:

PROVA SCRITTA 1

Residues Study

Determination of residues of AAA in rice following spray application in Southern Europe.

Summary

Two decline trials and two at-harvest trials were conducted in Southern Europe to determine residues of AAA in rice (grain with hulls, grain without hulls, hulls and straw) following a single application of the product XXX in 2017 at 60 days before normal harvest.
XXX is an emulsifiable concentrate (EC). Applications were made at a nominal rate of 22.5 g as/ha of AAA. The applications were made at between 57 and 61 days before normal commercial harvest.
All samples of rice grain with hull and straw were placed in freezers within 8 hours of sampling and transported frozen. Samples were stored in a freezer set to maintain a sample temperature of <-18°C.
Residues of AAA in rice (grain with hulls, grain without hulls, hulls and straw) were measured according to analytical method with a limit of quantification of 0.01 mg/kg.
The maximum period of frozen storage until extraction and analysis was 344 days for AAA.

Residues of AAA in rice are summarised below. The EU MRL for rice is 0.01 mg/kg.

Table 1: Proposed use for rice

Region	Application Method	Application Rate (kg a.s./ha)	Water Rate (L/ha)	Maximum No. of Applications	Application Interval (days)	Growth Stage (BBCH)	Min PHI (days)
EU South	Foliar spray, outdoor	30	2.5	1	n/a	BBCH 12 to 45	60

Study results

Trial No. Location (region)	Commodity/ Variety	Date of 1) Sowing or Planting 2) Flowering 3) Harvest	Method of Treatment	Application rate per treatment			No. of trt(s)	Dates of treatments	Growth stage at treatment	Portion analysed (commodity)	Residues (mg/kg)	PHI	Mean Recovery
				g as/hL	Water (L/ha)	g as/ha					A		
CEMS-8062A Alcácer do Sal, 7580-319, Setúbal, Portugal	Paddy Rice/ Cirio	1) 10 May 17 2) Aug 17 3) Oct 17	Broadcast application using a pump tank sprayer	11.3	205.6	23.178	1	07 Aug 17	56	Grain with Hull Grain without Hull Straw	ND ND 0.028	57 57 57	Grain with Hull: 93% Grain without Hull: 102% Straw: 95%

CEMS-8062A Alcácer do Sal, 7580-319, Setúbal, Portugal	Paddy Rice/ Jota Sendra	1) 09 May 17 2) Sep 17 3) Oct-Nov 17	Broadcast application using a knapsack sprayer	11.3	205.0	23.156	1	31 Jul 17	29-49	Grain with Hull Grain without Hull Straw	ND ND 0.035	59 59 59	Grain with Hull: 97% Grain without Hull: 100% Straw: 92%
CEMS-8062B Amposta E-43870, Tarragona, Spain	Paddy Rice/ Jota Sendra	1) 09 May 17 2) Sep 17 3) Oct-Nov 17	Broadcast application using a knapsack sprayer	15.1	193.3	29.125	1	31 Jul 17	29-49	Grain with Hull Grain without Hull Straw	ND ND 0.045	59 59 59	Grain with Hull: 97% Grain without Hull: 100% Straw: 92%
CEMS-8062C Navalvillar de Pela E-06760, Badajoz, Spain	Paddy Rice/ Gladio	1) 13 May 17 2) 12-16 Aug 17 3) 01-10 Oct 17	Broadcast application using a knapsack sprayer	45.0	53.3	24	1	01 Aug 17	45	Grain with Hull Grain without Hull Straw	ND ND ND ND ND 0.145 0.113 0.151 0.098	53 61 66 73 61 53 61 66 73	Grain with Hull: 97% Grain without Hull: 100% Straw: 92%
CEMS-8062C Navalvillar de Pela E-06760, Badajoz, Spain	Paddy Rice/ Gladio	1) 13 May 17 2) 12-16 Aug 17 3) 01-10 Oct 17	Broadcast application using a knapsack sprayer	60.0	52.2	31.33	1	01 Aug 17	45	Grain with Hull Grain without Hull Straw	ND ND ND ND ND 0.144 0.098 0.147 0.043	53 61 66 73 61 53 61 66 73	Grain with Hull: 97% Grain without Hull: 100% Straw: 92%
CEMS-8062D Caltignaga (NO) 28010, Piedmont, Italy	Paddy Rice/ Ronaldo	1) 15 May 17 2) 03-08 Aug 17 3) mid Oct 17	Broadcast application using a boom sprayer	15.0	189.52	28.43	1	28 Jul 17	65	Grain with Hull Grain without Hull Hulls Straw	(0.009) (0.009) 0.012 (0.009) ND ND ND 0.038 0.025 0.048 0.049 0.050 0.042	54 60 67 75 60 67 75 67 75 54 60 67 75	Grain with Hull: 97% Grain without Hull: 100% Hulls: 98% Straw: 92%

Conclusion:

PROVA SCRITTA 2

DOMANDE A SCELTA MULTIPLA (UNA SOLA RISPOSTA ESATTA)

1. I Principi Uniformi

1. Sono i criteri per l'etichettatura dei Prodotti Fitosanitari
2. Sono i criteri per la valutazione e l'autorizzazione dei pesticidi
3. Sono i criteri per la classificazione dei pesticidi
4. Sono i criteri per l'esclusione dei pesticidi tossici
5. Sono i criteri per individuare pesticidi cancerogeni e mutageni

2. Secondo il Regolamento CE 1107/2009, per essere autorizzato un principio attivo deve avere:

1. Caratteristiche non percolanti, basso impatto per lavoratori e astanti, essere poco tossico per le api, efficace, di chiaro beneficio per la produzione vegetale.
2. Elevata efficacia, bassa tossicità per organismi acquatici e artropodi, poco tossico per l'uomo, chiaro beneficio per la produzione vegetale
3. Dimostrata efficacia, assenza di effetti sulla salute umana, basso impatto ambientale, chiaro beneficio per la produzione vegetale
4. Elevata efficacia, assenza di effetti cancerogeni, mutageni e genotossici, bassa tossicità ambientale
5. Dimostrata efficacia, non creare rischi inaccettabili per l'ambiente, nessun effetto nocivo su salute umana, animale e sulle acque sotterranee, chiaro beneficio per la produzione vegetale

3. Il NOAEL (No Observable Adverse Effect Level)

1 È l'effetto tossico meno grave
2 È la dose più alta alla quale non si osservano effetti avversi nell'uomo
3 È la dose più bassa alla quale non si osservano effetti tossici
4 È la dose più alta alla quale non si osservano effetti avversi
5 È qualsiasi dose alla quale non si osservano effetti tossici

4 Il regolamento CLP prevede che i cancerogeni (indicare la risposta sbagliata):

1. Siano etichettati con il pittogramma con l'uomo che implode
2. Facciano parte delle sostanze CMR
3. Siano assegnate le frasi H 350 o H351
4. Tutte le precedenti risposte sono vere
5. Siano considerati genotossici

5 Quale dei seguenti non è un limite basato sulla salute

1. Acceptable daily intake (ADI)
2. Threshold Limit Value (TLV)
3. Maximum Residue Level (MRL) (Limite Massimo di Residuo (LMR)
4. Acceptable Operator Exposure Level (AOEL)
5. Derived No Effect Level (DNEL)

6 I criteri di cut-off secondo il Regolamento CE 1107/2009,

1. Si applicano prima di effettuare la valutazione di rischio di un principio attivo
2. Si applicano per identificare i principi attivi a basso rischio
3. Si applicano per identificare i prodotti fitosanitari a basso impatto ambientale
4. Si applicano per identificare i principi attivi "sicuri"

5. Si applicano dopo aver effettuato la valutazione di rischio di un principio attivo

7 La valutazione di rischio ambientale è

1. Un processo che valuta la pericolosità di una sostanza prima che questa venga immessa in ambiente e che permette la sua classificazione.
2. Un processo che valuta gli effetti che una sostanza può provocare nelle specie non bersaglio.
3. Un processo che valuta la probabilità che un determinato effetto ecologico avverso possa verificarsi (o si stia già verificando) a seguito di un'esposizione ad uno o più stress chimici, fisici, o biologici.
4. Un processo che valuta la distribuzione di una sostanza nei diversi comparti ambientali
5. Un processo che tiene conto dell'analisi costi/benefici prima di autorizzare all'uso un prodotto fitosanitario

PROVA SCRITTA 2

Ecotoxicology Study

Reference: CA 8.2.2.1/01	**Report:**	Doe, S.L. (2012); XX Technical (98% w/w): Early life-stage toxicity test with the sheepshead minnow, *Cyprinodon variegatus*, under flow-through conditions **DuPont Report No.:** DuPont-34270 **Guidelines:** OPPTS 850.1400 (1996), OECD 210 (1992) **Deviations:** None **Testing Facility:** ABC Laboratories, Inc. (Missouri), Columbia, Missouri, USA **Testing Facility Report No.:** 68029 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The early life-stage toxicity of XX to fed Sheepshead Minnow (*Cyprinodon variegatus*) was determined in a 29-day post-hatch flow-through test. The test was conducted in accordance with the U.S. EPA, Office of Prevention, Pesticides and Toxic Substance (OPPTS), Ecological Effects Test Guideline 850.1400 and the Organization for Economic Cooperation and Development (OECD), Guideline 210. Treatments consisted of a dilution water control and six nominal concentrations of 0.025, 0.050, 0.10, 0.20, 0.40, and 0.80 mg a.s./L. Based on mean measured concentrations of XX, the NOEC value for egg hatchability, post-hatch survival, standard length, and blotted wet weight was 0.356 mg a.s./L. The most sensitive endpoints were egg hatchability, fry survival and fry growth, with an MATC value of 0.506 mg a.s./L.

MATERIALS AND METHODS

A. MATERIALS

1. Test material: Pure XX (PAI)
   Lot/Batch #: D1410-196
   Purity: 98.0% by analysis
   Description: White crystalline solid
   CAS#: -
   Stability of test compound: Stable at normal temperatures and storage conditions
2. Control: Dilution (laboratory blended water) water
   Solvent control: None
   Test vehicle: Dilution water (laboratory salt water)
   Toxic reference: None
3. Test organism: Sheepshead Minnow
   Species: *Cyprinodon variegatus*
   Age at dosing: <24 hours
   Initial population: 20 embryos per test chamber

Source:	In-house culture
Diet:	Brine shrimp nauplii and/or salmon starter at least twice daily except 24 hours prior to termination
Test chamber:	Glass aquaria measuring approximately 15 cm wide by 22 cm long by 24 cm high with a test solution depth of 14 cm

4. Environmental conditions (in-life period)

Temperature:	24.1 to 24.9°C for fry
Dissolved oxygen concentration:	6.0 to 7.4 mg/L (83 to 101% saturation)
pH:	8.1 to 8.3
Salinity:	19.6 to 21.7‰
Photoperiod:	16 hr photoperiod (643 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

   28-March-2012 to 05-May-2012

2. Experimental treatments

   The early life-stage toxicity of XX to fed *Cyprinodon variegatus* was determined in an unaerated, flow-through, 29-day post-hatch test. Treatments consisted of a dilution water control, and six nominal concentrations of 0 (control), 0.025, 0.050, 0.10, 0.20, 0.40, and 0.80 mg a.s./L. Twenty embryos were used per replicate with four replicates per test concentration and control.

3. Observations

   On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. Survival of hatched fry was monitored daily by visually inspecting each test chamber and any behavioral or physical changes, including abnormalities, were recorded. At the end of the 29-day post-hatch exposure, all surviving fry were measured for standard length (*i.e.*, tip of the snout to the caudal peduncle) using a millimeter scale and blotted wet weight using an electronic balance.

   Temperature, pH, and dissolved oxygen concentration were measured in all replicates of the test substance treatments and control groups at test initiation, weekly throughout the test, and at termination of the definitive test. A continuous recording of temperature was made using a data logger and thermistor probe placed in a centrally-located test chamber. The concentration of XX was measured in test solution samples collected from the control and each treatment prior to the definitive test initiation (Day –1), and on Days 0, 7, 14, 21, 28, 31, 35, and 38 of the definitive test. The analysis of the samples for XX during the test was based on an analytical method provided by the Sponsor and validated at ABC Laboratories prior to the definitive test initiation.

4. Statistics

   The NOEC for egg hatchability and fish survival (29-day post-hatch) data were determined by using a Fisher's exact test. A Hochberg adjustment was used to control the experiment-wise error rate for the Fisher's test at the same alpha level. In addition, the NOEC for these parameters was estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test with the alternate hypothesis being the mean for the parameter was reduced in comparison to the control mean. The time to start of hatch and time to complete hatch was evaluated using an ANOVA procedure and a one-tailed Dunnett's test with the alternate hypothesis being the mean for the parameter was greater in comparison to the control mean. The NOEC values for standard length and blotted wet weight, were determined using a nested ANOVA procedure, where the treatment means are weighted for number of fish in each chamber, and a one-tailed Dunnett's test with the alternate hypothesis being the mean for the parameter was reduced in comparison to the control mean. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments. When the Shapiro-Wilk's and Levene's tests indicated normality and homogeneity of variance the non-transformed data were used in the parametric ANOVA and Dunnett's test. The transformed (*i.e.*, ranks) data were used when the Shapiro-Wilk's and Levene's tests indicated non–normality and/or heterogeneity of variance. Where possible, the point estimates of the maximum acceptable toxicant concentration (MATC) were calculated as the geometric mean of the NOEC and LOEC values of the sensitive endpoints.

RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of XX in the control and test substance treatments during the study were <LOD (control), 0.0198, 0.0424, 0.0826, 0.169, 0.356, and 0.718 mg a.s./L, which represented 78 to 90% of the nominal concentrations. No residues of XX were detected in the control above the LOD of 0.00000537 mg a.s./L. All test acceptability criteria were met. Egg hatch began in the control and the 0.0198, 0.0424, 0.0826 mg a.s./L treatments on study Day 5. Hatch in the 0.169, 0.356, and 0.718 mg a.s./L treatments began on study days 6, 6, and 7, respectively. Day 0 post-hatch (*i.e.*, ≥95% hatch) in the control treatment was determined to be Day 9. Hatch was completed in all treatment replicates between study Days 9 and 13, with the exception of one replicate in each of the 0.0826, 0.169, and 0.356 mg a.s./L treatments where complete hatch was achieved on study Days 15, 14, and 16, respectively. Overall hatching success in the control treatment was 86% which met the acceptability criterion for this endpoint. Hatching success in the test substance treatments ranged from 68% in the 0.718 mg a.s./L treatment to 81% in the 0.0198 and 0.0424 mg a.s./L treatments. There was no statistically significant delay in time to start or completion of hatch observed in the test substance treatments as compared to the control. There was a statistically significant reduction in hatch success observed in the 0.718 mg a.s./L test substance treatment as compared to the control. Post-hatch survival in the control treatment was 93% which met the acceptability criterion for this endpoint.

Mean standard length in the control and test substance treatments was 14.6, 14.7, 15.5, 15.2, 14.4, 14.0, and 8.2 mm in the control, 0.0198, 0.0424, 0.0826, 0.169, 0.356, and 0.718 mg a.s./L treatments, respectively. Mean blotted wet weight in the control and test substance

treatments was 0.1099, 0.1100, 0.1228, 0.1278, 0.1074, 0.0997, and 0.0267 g in the control, 0.0198, 0.0424, 0.0826, 0.169, 0.356, and 0.718 mg a.s./L treatments, respectively. There was a statistically significant reduction of mean standard length and mean blotted wet weight at the 0.718 mg a.s./L treatment as compared to the control.

A summary of hatching and survival is presented in the following table.

Table 1: Summary of observed mortality of *Cyprinodon variegatus* exposed to XX in a flow-through test

Mean measured XX concentration (mg a.s./L)	Hatch (no. of hatched fry/initial no. of embryos)				Survival (no. of surviving fry/total no. of hatched fry)			
	A	B	C	D	A	B	C	D
Control	19/20	17/20	17/20	16/20	17/19	17/17	17/17	13/16
0.0198	15/20	14/20	17/20	19/20	14/15	10/14	14/17	18/19
0.0424	18/20	17/20	16/20	14/20	15/18	17/17	15/16	14/14
0.0826	13/20	16/20	15/20	14/20	10/13	14/16	10/15	14/14
0.169	16/20	13/20	16/20	16/20	14/16	11/13	14/16	14/16
0.356	19/20	16/20	15/20	14/20	16/19	16/16	15/15	11/14
0.718	13/20	13/20	13/20	15/20	7/13	9/13	8/13	7/15

Table 2: Effect Concentrations for Hatchability, Post-Hatch Survival, Length, and Weight of Sheepshead Minnow (*Cyprinodon variegatus*) Exposed to XX

Biological Parameter	No-Observed-Effect Concentration (NOEC) [a]	Lowest-Observed-Effect Concentration (LOEC)	MATC
Day Hatch Start	0.718	>0.718	NA
Day Hatch Completed	0.718	>0.718	NA
Egg Hatchability	0.356	0.718	0.506
Fry Survival [b]	0.356	0.718	0.506
Standard Length	0.356	0.718	0.506
Blotted Wet Weight	0.356	0.718	0.506

[a] Expressed as mean measured concentration of oxamyl (mg a.s./L).
[b] Fry survival based on number of hatched fry surviving on day 29 post-hatch.
NA = Not applicable

CONCLUSION

Based on mean measured concentrations of XX, the NOEC value was 0.356 mg a.s./L based on egg hatchability, post-hatch survival, standard length, and blotted wet weight.

Comments:

PROVA SCRITTA 2

Fate and behaviour in the environment Study

Reference:	CA 7.1.1.1/03
Report:	Doe, D. C., Hoe, C. L. (2003) XXX – Paddy Soil Metabolism and Degradation Rate Study, under laboratory conditions Syngenta Jealott's Hill International Research Centre, Bracknell, UK Report No RJ3361B
Guideline(s):	JMAFF Test Guidelines Noshan No 8147, 24 November 2000
GLP:	Yes

Executive Summary

The degradation of XXX was investigated in a flooded Japanese paddy soil (Ushiku sandy loam). Anaerobic soil conditions were established by flooding the soil and incubating under a stream of air. Once soil anaerobicity was achieved after 43 days of pre-incubation, ^{14}C-phenyl labelled XXX was applied to the water phase of the systems at a rate of 0.475 mg a.s./kg soil, equivalent to 0.71 kg a.s./ha (based upon a 10 cm soil layer and a soil density of 1.5 g/cm^3), and incubated in the dark, at 25 ± 3 ºC, for up to 119 days.

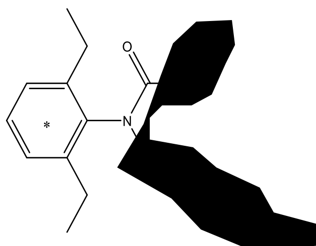
Duplicate samples were collected immediately after application and at 4 and 95 DAT, and single samples were collected at 7, 14, 28, 56 and 119 DAT. Following sampling, water was decanted from the test vessels and the water and soil phases were analysed separately. Radioactivity in the water phase was quantified by LSC and characterised by HPLC. Soils were extracted by a variety of solvents and conditions. Initially simple solvent extractions were carried out at room temperature, followed by solvent extractions with mild acidification at room temperature. If high levels of unextracted material remained, then Accelerated Solvent Extraction (ASE) with elevated temperature and pressure were employed. Extracts were analysed by LSC to determine the amount of radioactivity present and characterised and quantified by HPLC. The LOD and LOQ of the methods was not reported.

The measured redox potentials for soil gave values of 100 – 200 mV for the duration of the study following test item application, which demonstrated soil anaerobicity. Paddy water redox measurements were approximately 220 mV to 430 mV (values derived from graphical output presented in study report) demonstrating that the paddy water was predominantly aerobic following application of the active substance.

Mass balances were between 92.9 and 104.0% AR, with the exception of 56 DAT, which had a recovery of 87.6% AR. XXX rapidly dissipated from the water phase, principally by adsorption to the soil to a maximum of 38.6% AR at 14 DAT, then degraded to CGA80154 (max 5.6% AR at 28 DAT), CGA86903 (max 9.4% AR at 95 DAT – mean of two replicates) and unextractable residues (max 58% AR at 119 DAT). Low levels of other extractable compounds were observed, but no single metabolite amounted to more than 3.5% AR. Total volatiles reached a maximum of 1.1% AR over the study duration.

Materials

Test substance (material): **[Phenyl-U-^{14}C]-XXX**



*shows the radiolabel position

Lot/Batch #: ILA-138.1-1
Specific activity: 2.21 MBq/mg
Radiochemical Purity: 99.1% (determined by HPLC prior to application)
Supplier: Syngenta Crop Protection AG, Basel, Switzerland

Soil Properties:

Name	**CRS Ushiku Paddy H**
Batch	NRM-0533/02
Sampling location	Syngenta Japan KK, 780 Kuno-cho, Ushiku-shi, Ibraki 300-1147, Japan
Date of collection	18th March 2002
Sampling depth (cm)	5-20
Collection procedures	Not reported
Pesticide history	No applications of pesticides for at least five years
Storage conditions	Shipped to the laboratory on blue ice. Stored in a loosely folded plastic bag at 4 ºC
Duration of storage	Not reported
Soil preparation	Sieved (2 mm)
Particle size (% w/w) :	
Clay (<2 µm)	19
Silt (50-2 µm)	20
Sand (2000-50 µm)	61
Texture (USDA)	Sandy loam
pH (water)	5.8
pH ($CaCl_2$)	5.2
Organic matter (%)	6.2
Organic carbon (%)*	3.48
CEC (meq/100 g soil)	23.4
Water holding capacity at 0.33 Bar (% w/w)	30.2
Water holding capacity at 15 bar (% w/w)	15.1
Biomass (mg C 100 g^{-1})	42.68

*Organic carbon (OC) % = organic matter (OM) %/1.724. CEC = cation exchange capacity.

Study Design and Methods

Experimental design

Parameter	Description
Duration of the test	Acclimatisation: 43 days Incubation: 119 days
Soil condition	Moist soil, passed through 2 mm sieve prior to use

Parameter		Description
Soil sample weight		200 g (dry soil) + 250 mL Ultra-pure water per replicate
Test concentration* (mg test item/kg soil (dry weight))		0.475 (95.0 µg per test vessel)
Number of replicates		2 at DAT 0, 4, 95. 1 for all other sample intervals.
Test apparatus		Glass incubation bottle with a constant stream of air
Volatile traps		1 x ORBO 100 (Carbotrap 20/40) to capture 5 to 12 carbon units, 1 x ORBO 91 (Carbosieve S-III) to capture 2 to 5 carbon units, 2 x ethanolamine to capture $^{14}CO_2$
Test material application	Identity of solvent	acetonitrile:methanol
	Volume of test solution used/treatment	100 µL
	Application method	Pipette
	Evaporation of application solvent	No
Experimental conditions	Temperature (°C)	25±2
	Continuous darkness (Yes/No):	Yes

*Equivalent to an application rate of 0.71 kg a.s/ha assuming a 10 cm soil layer with bulk density of 1.5 g/cm^3

Sampling

Parameter	Description
Sampling intervals	Duplicate samples were taken at: 0, 4, 95 DAT Single samples were taken at: 7, 14, 28, 56, 119 DAT
Sampling procedures	Water was decanted from test vessels. Volatile traps were replaced with fresh solutions at each sampling interval (except for 4 DAT).
Parameter measurements	Redox potentials of the soil were taken at approximately 4-8 day intervals during the equilibration period and at every sampling interval during the test incubation.
Sample storage before analysis	Samples were stored at -20ºC. Water and soil extracts were quantified by LSC on the day of sampling and analysed by HPLC within 3 days. For 95 and 119 DAT, samples were combined, concentrated and reanalysed up to 4 months later. The stability of XXX and the metabolites to these storage conditions was shown to be valid for at least 8 and 6 months, respectively.

Description of analytical procedures

Soil extraction:

Soil Extraction Step	Description
Extraction A	Methanol (100 mL), 30 mins on wrist action shaker, followed by centrifugation, 100 or 2000 RPM, 3 mins.
Extraction B	70:30 Methanol: water pH 2, (80 mL), 30 mins on wrist action shaker, followed by centrifugation 2000 RPM, 4 mins.
Extraction C	50:50 Acetonitrile: water pH 2 (80 mL), overnight on a flat bed shaker, followed by centrifugation 2000 RPM, 4 mins
Extraction D	70:30 Acetonitrile: water pH 2 (80 mL), 30 mins on a wrist action shaker, followed by centrifugation 2000 RPM, 5 mins

Extraction E	50:5 Acetonitrile: UAA(aq), 60 mins on a wrist action shaker, followed by centrifugation 2000 RPM, 5 mins
Extraction F	Accelerated solvent extraction (ASE) – 70:30 acetonitrile: water, 100°C, 1500 psi, followed by 70:30 acetonitrile: 0.3 M formic acid, 100°C, 1500 psi.
Extraction G	Accelerated solvent extraction (ASE) – 70:30 acetonitrile: waer, 100°C, 1500 psi, followed by 70:30 acetonitrile: water, 100°C, 1500 psi.
Additional extraction using reflux under neutral, acidic and basic conditions were explored, however the parent material was not stable to the extraction conditions or the extraction process was unsuccessful.	
28 and 119 DAT samples were further extracted to determine the radioactivity associated with the humic and fulvic acid and humin soil organic matter fractions.	

DAT	Extraction Number or Type						
	1st	2nd	3rd	4th	5th	6th	ASE
0	A	B	B	-	-	-	-
4	A	B	B	-	-	-	-
7	A	B	B	C	D	E	-
14	A*	B*	B*	C	D	E*	-
28	A	B	B*	D*	-	-	F
56	A*	B*	B*	D*	E*	-	F
95	A*	B*	B*	-	-	-	G
95	A*	A*1	-	-	-	-	G
119	A*	B*	B*	-	-	-	G

*These extracts were combined with each other (for each time point) and used for quantification (HPLC only).
[1]This extraction only used 80 mL not 100 mL.

Samples analysis:

Matrix	Description
Water	The volume of decanted flood water was measured and the radioactivity quantified by LSC and HPLC. If necessary, samples were concentrated, up to a maximum of 15 times the original concentration, before LSC and HPLC analysis.
Soil extracts	The volume of each extract was measured and the radioactivity quantified by LSC and HPLC with radiodetection. Where necessary, extracts were combined and concentrated to allow quantitative analysis (as shown in the table above).
Unextractable soil residues	Extracted soils were air dried and finely ground. The radioactivity in the soil was quantified by combustion using either a Harvey OX300 Biological Oxidiser or a Packard 387 Automated Biological Oxidiser. Evolved $^{14}CO_2$ was trapped in Optiphase Safe: 2-methoxyethylamine: water (50:25:2, v:v:v) and quantified by LSC.
Volatiles	On removal, the radioactivity in the ethanolamine traps was quantified by LSC. Carbon sieves were extracted for 28 and 56 DAT sampling points by passing approximately 10 ml of acetonitrile through each. The radioactive content was determined by LSC.

Analytical methods:

Method	Description

LSC	Radioactivity in samples were quantified by LSC using a Wallac 1409 scintillation spectrometer.
HPLC	The purity of the test substance and characterisation of radioactivity in water and soil extracts was determined using an Agilent 1100 System with a UV detector (215 nm) and coupled to a radiodetector (Packard Radiomatic TM525 TR Flow Scintillation Analyser)
GC-MSD	Gas chromatography was used to confirm the presence or absence of XXX and metabolites from concentrated soil extracts using an Agilent 5973 MSD system.
HPLC-MS/MS	HPLC-MS/MS was used to confirm the presence or absence of XXX and metabolites from concentrated soil extracts using an Agilent 1100 system.
TLC	Reversed phase thin layer chromatography was used to confirm qualitatively that only parent material was present in the application solution using Merck RP18F254 plates with chloroform: acetone (9:1, v/v) and chloroform: methanol: formic acid: water (75:20:4:2, v/v/v/v) solvent systems.

Results and Discussion

The measured redox potentials for soil gave values of 100 – 200 mV for the duration of the study following test item application, which demonstrated soil anaerobicity. Paddy water redox measurements were approximately 220 mV to 430 mV (values derived from graphical output presented in study report) demonstrating that the paddy water was predominantly aerobic following application of the active substance. The distribution and characterisation of radioactivity in the paddy system are shown in **Table 8. 1**. Radioactivity recovered from the total systems was 102 to 104% of the applied dose at 0 DAT. Recoveries of radioactivity from 56 DAT was 87.6% AR but were between 92.9% AR and 104.0% AR for all other samples.

XXX dissipated from the water phase to the soil, reaching a maximum of 39% AR in soil extracts at 14 DAT, then declining to 8% AR by 119 DAT. Unextracted radioactivity in the soil residues increased throughout the incubation period to 58% AR by 119 DAT. A total of 1% AR was collected as volatiles during the 119 day incubation (both in the CO_2 traps and the carbon sieves). CGA80154 and CGA86903 were observed at maxima of 5.6% AR at 28 DAT and 9.4% AR (mean of two replicates) at 95 DAT, respectively. Low levels of other extractable compounds were observed, but no single metabolite amounted to more than 3.5% AR.

Table 8. 1: Distribution and characterisation of radioactivity in the paddy system treated with ^{14}C-phenyl labelled XXX (as % applied radioactivity)

	Incubation time (days)										
	0	**0**	**4**	**4**	**7**	**14**	**28**	**56**	**95**	**95**	**119**
Water Phase											
XXX	96.8	97.6	66.1	60.7	52.6	17.9	9.9	2.9	<LOD	<LOD	<LOD
CGA80154	-	-	-	-	-	-	-	-	-	-	-
CGA86903	-	-	-	-	-	-	0.4	-	-	-	-
Others	0.7	3.1	0.7	1.3	1.3	1.7	0.6	3.2	3.9	3.3	6.3
Total	**97.5**	**100.7**	**66.8**	**62.0**	**53.9**	**19.6**	**10.9**	**6.1**	**3.9**	**3.3**	**6.3**
Soil Extracts											
XXX	3.6	2.4	16.0	18.3	27.7	38.6	27.7	31.7	13.4	6.9	7.6
CGA80154	-	-	-	-	0.1	0.6	5.6	5.2	5.5	2.7	3.8
CGA86903	-	-	0.2	-	-	0.8	4.1	2.5	6.4	12.3	8.3
CGA80156	-	-	-	-	-	-	-	-	-	-	3.5
Others	0.6	0.9	0.6	0.8	1.5	2.3	5.3	8.0	13.1	15.6	12.7
Total	**4.2**	**3.3**	**16.8**	**19.1**	**29.3**	**42.3**	**42.7**	**47.4**	**38.4**	**37.5**	**35.9**
Total system											
$^{14}CO_2$*	n/a	n/a	<0.1	<0.1	<0.1	0.3	0.3	0.5	0.5	0.6	1.1
XXX	100.4	100.0	82.1	79.0	80.3	56.5	37.6	34.6	13.4	6.9	7.6
CGA80154	<0.1	<0.1	<0.1	<0.1	0.1	0.6	5.6	5.2	5.5	2.7	3.8
CGA86903	<0.1	<0.1	0.2	<0.1	<0.1	0.8	4.5	2.5	6.4	12.3	8.3
CGA80156	-	-	-	-	-	-	-	-	-	-	3.5
Others	1.3	4.0	1.3	2.1	2.8	4.0	5.9	11.2	17.0	18.9	19.0
Unextracted	n/a	n/a	19.2	15.6	15.7	34.3	46.6	33.6	50.1	56.6	58.2
Total	**101.7**	**104.0**	**102.8**	**96.7**	**98.9**	**96.5**	**100.5**	**87.6**	**92.9**	**98.0**	**101.5**

* Cumulative
Others = unidentified radioactivity including baseline material i.e. not associated with XXX or the metabolite standards and no single component accounting for >3.5% AR
n/a = not applicable
A value of 0.0 represents either not detected or <0.05%

Conclusions

XXX rapidly dissipates from the water, principally by adsorption to the soil, then degrades to CGA80154 (max 5.6% AR), CGA86903 (max 9.4% AR) and unextractable residues (max 58% AR).

Comments:

PROVA SCRITTA 2

Mammalian toxicology Study

In a 13-week study, compound X (purity 93.4%) was administered to four groups of 7-week-old CD-1 mice (12 mice of each sex per group) at a dietary concentration of 0, 1750, 3500 or 7000 ppm (equal to 0, 204, 405 and 807 mg/kg bw per day for males and 0, 252, 529 and 1111 mg/kg bw per day for females, respectively). Achieved concentrations and homogeneity were verified by chemical analysis. All animals were observed for viability/mortality twice daily. Clinical signs were recorded once daily during the treatment. Feed consumption and body weights were recorded weekly. Blood samples were taken at termination of the study for haematology and clinical chemistry. All animals were subjected to detailed postmortem gross examination. Organ weights (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes and epididymides, thymus, uterus and cervix) were recorded, and an extensive list of tissues was examined histopathologically.

No mortality or clinical signs were reported during the study. No changes on feed consumption were reported. No body weight or body weight gain were reported in males. Small, not statistically significant reductions in body weight gain were recorded in females given 1750 and 3500 ppm; the reduction was statistically significant in females given 7000 ppm.

Statistical analysis revealed the following statistically significant changes: decreases in haemoglobin concentration and packed cell volume in males given 3500 ppm, and increased platelets in males given 1750 and 7000 ppm. However, these effects were not dose related and in general were within or close to historical control ranges, and therefore they were not considered to be treatment related. The statistically significant dose-responsive reduction in mean cell haemoglobin concentration noted in males. In clinical chemistry, a significant decrease in mean glucose was noted in 7000 ppm females. Dose-responsive changes were noted for chloride, urea and total bilirubin.

Increases in absolute and relative liver weights were noted in both sexes of all treated groups; however, these increases were not accompanied by any clinical chemistry or histopathological findings. No other findings were noted.

Key findings of the 13-week dietary toxicity study in mice

	Males					**Females**				
Parameter	**0 ppm**	**1 750 ppm**	**3 500 ppm**	**7 000 ppm**	**HCD[a] (mean; range) or DR**	**0 ppm**	**1 750 ppm**	**3 500 ppm**	**7 000 ppm**	**HCD[a] (mean; range) or DR**
Body weight gain, weeks 0–13 (g)	10.5	9.6	8.9	9.7	–	8.3	7.5	7.4	6.1*	–

Parameter	Males					Females				
	0 ppm	1 750 ppm	3 500 ppm	7 000 ppm	HCD[a] (mean; range) or DR	0 ppm	1 750 ppm	3 500 ppm	7 000 ppm	HCD[a] (mean; range) or DR
Feed consumption, mean, weeks 0–13 (g/mouse per day)	5.2	5.0	4.9	5.0	–	4.6	4.5	4.8	4.9	–
Initial body weight (g)	33.1	34.1	33.8	34.7	–	25.2	24.8	24.7	24.6	–
Terminal body weight (g)	43.6	43.7	42.7	44.4	–	33.5	32.3	32.1	30.7	–
Haematology										
Hb (g/dL)	14.6	14.3	13.2**	14.1	13.4; 12.0–15.1	14.6	14.9	14.8	14.1	–
PCV (%)	44.8	44.6	41.6*	44.9	44.5; 38.0–50.4	45.1	45.8	45.8	44.3	–
PLT (10^9/L)	1 292	1 756**	1 450	1 666*	1 390; 941–1 721	1 555	1 351	1 390	1 526	–
MCHC (g/dL)	32.3	32.1	31.7	31.5	DR*	32.4	32.5	32.3	31.9	–
Clinical pathology										
GLUC (mmol/L)	8.9	9.7	9.3	9.1	–	10.8	10.3	9.3	9.1*	9.7; 7.9–12.0
Cl (mmol/L)	109	109	109	107	DR*	112	112	112	112	–
Urea (mmol/L)	6.4	6.3	6.7	7.3	DR*	7.1	6.8	6.6	6.6	–
T Bili (µmol/L)	2.5	1.8	2.1	1.8	DR*	1.7	1.7	1.7	1.9	–
Organ weights										
Body weight at necropsy (g)	44.0	44.6	43.3	45.0	–	33.9	33.3	32.7	31.9	–
Absolute liver weight (g)	2.00	2.11	2.14	2.35***	–	1.58	1.74	1.64	1.81	–
% change	–	*5.5*	*6.9*	*17.4*	–	–	*10.1*	*3.9*	*14.5*	–
Relative liver weight (%)	4.55	4.73	4.95**	5.22***	–	4.65	5.20*	5.02	5.66***	–
% change	–	*4.0*	*8.8*	*14.7*	–	–	*11.8*	*8.0*	*21.7*	–
Histopathology of liver										
No. examined	*12*	*12*	*12*	*12*	–	*12*	*12*	*12*	*12*	–
Focal necrosis	2	1	4	5	–	3	2	6	3	–
Inflammatory cell foci	11	11	11	11	–	12	12	12	12	–
Mitotic figures	0	1	0	0	–	0	0	0	2	–
Hepatocyte vacuolation	0	0	0	0	–	0	1	0	0	–
Glycogen vacuolation	8	8	7	9	–	12	10	11	12	–

Parameter	Males					Females				
	0 ppm	1 750 ppm	3 500 ppm	7 000 ppm	HCD[a] (mean; range) or DR	0 ppm	1 750 ppm	3 500 ppm	7 000 ppm	HCD[a] (mean; range) or DR
Pigmented histiocytes	0	2	0	0	–	0	1	0	1	–
Agonal congestion/ haemorrhage	0	0	1	0	–	0	2	0	0	–
Haematopoiesis	0	1	0	0	–	0	0	0	0	–

Cl: chloride; DR: dose–response test; GLUC: glucose; Hb: haemoglobin; HCD: historical control data; MCHC: mean cell haemoglobin concentration; PCV: packed cell volume; PLT: platelets; ppm: parts per million; T Bili: total bilirubin; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (analysis of variance, dose–response and Dunnett's)

[a] Historical control data: five studies, 34 animals of same strain and age housed at this laboratory, from 2003.

Conclusion:

PROVA SCRITTA 2

Methods of Analysis Study

Description of analytical methods for the determination of relevant impurities in the product

The technical active substance contains ≤ 1 g/kg of IMPURITY A which is considered to be an impurity of toxicological concern.
The following analytical method has been developed for the determination of IMPURITY A in the formulation containing 13% of the technical active substance.

Report	Determination of the IMPURITY A in four containing ACTIVE SUBSTANCE Y formulations of Suspo-Emulsion (SE) formulation type. Method XXX
viations:	No
GLP:	No, not subject to GLP regulations
Acceptability:	Yes

Report	Complete validation of the method XXX for determination of IMPUTITY A in four containing ACTIVE SUBSTANCE Y formulations of Suspo-Emulsion (SE) formulation type.
Guideline(s):	SANCO/3030/99
Deviations:	No
GLP:	yes
Acceptability:	Yes

Materials and methods

This method covers the qualitative and quantitative determination of IMPURITY A in crop protection formulations containing the ACTIVE SUBSTANCE Y. The samples are analysed by gas chromatography with FID detection using external calibration. The results are calculated by peak area comparison with internal standard. The chromatographic conditions are summarised below:

Method parameters

Column	DB-5 30 m x 0.25 mm x 1.0 µm
Mobile phase	Helium
Column temperature	Start 30 °C hold 1 min. 5 °C/min up to 100 °C, 25°C/min up to 300°C hold 9 min.
Column flow	Start 1.5mL/min, with a rate of 0,5 mL/min up to 3.0 mL/min, hold for 24 min
Injection volume	1 µL
Injector temperature	240 °C
Injection mode	Pulsed Splitless (splitless for the first 2 minutes, then split 100:1)
Detector temperature	300°C
Approximate retention times	IMPURITY A (IMPURITY A) 13.6 minutes O-Xylene (ISTD) 15.2 minutes
Total running time	42 min.

Validation - Results and discussions

Principle of method	HS-GC with FID detection
Linearity **(linear between mg/L)** **(correlation coefficient, expressed as r)**	Linear equation. The sample response ratio between the analyte and the internal standard (quotient) results from the chromatograms are the y inputs. The x outputs are the calculated concentrations (in the same units used for the reference solutions Range: 4.72 mg/L – 30.195 mg/L equivalent to approx. 110 – 700 mg/kg IMPURITY A in the formulation R: 0.99968 Slope: 0.02567Intercept: -0.03810
Precision – Repeatability Mean **n = 5**	Mean: 0.0190 % ≙ 9.82 mg/L %RSD: 4.22
Accuracy **n = 3 (x2) at each level** **(% Recovery)**	Approx. 6.5 mg/L level (equivalent to approx. 145 mg/kg IMPURITY A in formulation) recovery range: 114.7 - 116.6 % mean recovery: 115.6% Recovery limit: 75 – 125 % Approx. 13.1 mg/L (equivalent to approx. 300 mg/kg IMPURITY A in the formulation) level: recovery range: 104.0 – 108.5 % mean recovery: 106.8) Recovery limit: 80 – 120 % Approx. 24.5 mg/L (equivalent to approx. 600 mg/kg IMPURITY A in BAS 657 01 H) level: recovery range: 101.5 –105.0 % mean recovery: 102.9 Recovery limit: 80 – 120 %
LOQ	LOQ: 10 x signal to noise level = 1 mg/L (22.3 mg/kg in the formulation)

Conclusion:

PROVA SCRITTA 2

Residues Study

Freezing storage stability of X and its metabolite X1 in rice.

Summary

The freezer storage stability of X and its metabolite X1 in rice grain (high starch content), rice straw (high water content) and rice whole plant (high water content) was investigated.
Samples were fortified with X or X1 at a rate of 0.2 mg/kg (20x LOQ) and were stored deep frozen (below -18°C). Samples were analysed after 0 and 299 days of storage in duplicate together with two freshly fortified samples acting as procedural recoveries and blank control samples.
Uncorrected mean recoveries of X in rice grain, straw and whole plant are 72, 96 and 93% of the initial level, respectively, after storage of 299 days, demonstrating the storage stability of X for at least 10 months in high starch and high water containing matrices. Uncorrected mean recoveries of X1 in rice grain, straw and whole plant are 98, 94 and 93% of the initial level, respectively, after storage of 299 days, demonstrating the storage stability of X for at least 10 months in high starch and high water containing matrices.

Table 1. Storage stability of X and X1 in fortified samples after freezer storage

Storage time [days]	Nomina l spiking level [mg/kg]	Residues after storage [mg/kg]	Uncorrected recoveries after storage [% of nominal]		Mean recoveries after storage [% of initial	Procedural recoveries [%] (spiked at 0.01 mg/kg) Procedural Recovery for Freshly Spiked Control Sample (%)	
			Single	Mean		Single	Mean
X - Rice whole plant							
0	0.2	0.197, 0.201	99, 101	100	—	102, 93	98
299	0.2	0.187, 0.183	94, 92	93	93		
X - Rice straw							
0	0.2	0.207, 0.188	104, 94	99	---	89, 101	95
299	0.2	0.192, 0.187	96, 94	95	96		
X - Rice grain							
0	0.2	0.188, 0.170	94, 85	90	---	100, 93	97
299	0.2	0.128, 0.129	64, 65	64	72		
X1 - Rice whole plant							
0	0.2	0.187, 0.210	94, 105	99	---	86, 94	90
299	0.2	0.180, 0.187	90, 94	92	93		
X1- Rice straw							
0	0.2	0.219, 0.206	110, 103	106	---	101, 92	97
299	0.2	0.204, 0.196	102, 98	100	94		
X1- Rice grain							
0	0.2	0.176, 0.203	88, 102	95	---	90, 82	86
299	0.2	0.189, 0.184	95, 92	93	98		

Conclusion:

PROVA SCRITTA 3

DOMANDE A SCELTA MULTIPLA (UNA SOLA RISPOSTA ESATTA)

1. **Secondo il Regolamento CE 1107/2009, una sostanza vPvB (very Persistent very Bioaccumulative)**
    1. Può essere autorizzata quando ha esposizione trascurabile
    2. Può essere autorizzata ma deve essere accompagnata da un monitoraggio specifico
    3. Va valutata caso per caso
    4. Può essere autorizzata in pochi casi particolari
    5. Non può essere autorizzata

2. **L'etichetta è il documento che racchiude tutta la valutazione effettuata per registrare un prodotto fitosanitario e, in Italia**
    1. Può essere in una lingua appartenente al Sud Europa perché ormai l'autorizzazione dei prodotti fitosanitari è zonale.
    2. Può essere in una lingua appartenente al Sud Europa perché si è applicato il mutuo riconoscimento.
    3. Può essere in una lingua dell'Unione Europea purché secondo CLP
    4. Deve essere in italiano con il numero di registrazione del Ministero della Salute.
    5. Deve essere nella lingua del paese produttore a patto che ci sia anche la traduzione italiana

3. **Secondo il Regolamento CE 1107/2009, un nuovo principio attivo è autorizzato all'uso**
    1. A seguito di una valutazione nazionale
    2. A seguito di una valutazione zonale
    3. A seguito della valutazione di EFSA
    4. A seguito della valutazione della Commissione Europea
    5. A seguito di una procedura Europea

4. **Un residuo di fitofarmaco non-conforme al LMR in un campione di riso è la conseguenza di?**
    1. Falsi negativi
    2. Errore nella diluizione del fitofarmaco
    3. Un TMDI maggiore di 100%
    4. Assenza di pioggia
    5. Contaminazioni

5. **Una sostanza attiva può essere autorizzata secondo il Regolamento CE 1107/2009,:**
    1. 10 anni se "normale", 15 anni se "a basso rischio", 7 anni se "candidata alla sostituzione"
    2. 10 anni se "normale", illimitata se "a basso rischio", 7 anni se "candidata alla sostituzione"
    3. 15 anni se "normale", 25 anni se "a basso rischio", 10 anni se "candidata alla sostituzione"
    4. 25 anni se "normale", illimitata se "a basso rischio", 17 anni se "candidata alla sostituzione"
    5. 5 anni se "normale", 10 se "a basso rischio", 3 anni se "candidata alla sostituzione"

6. **Gli studi di tossicità su mammiferi sono di norma:**

1. Solo a breve termine
2. Solo a lungo termine
3. Solo in-vitro e in-silico
4. Solo in-vivo ed ex-vivo
5. Sia *in-vitro* che *in-vivo*

7. Cosa si intende per NOEC?

1. La concentrazione più bassa testata a cui non si osservano effetti.
2. La dose di utilizzo a cui corrisponde l’assenza di concentrazioni ambientali
3. La concentrazione più alta testata a cui non si osservano effetti.
4. La dose a cui corrisponde una variazione di concentrazione di azoto (nitrogen) nel suolo.
5. La dose a cui corrisponde una concentrazione ambientale osservabile

PROVA SCRITTA 3

Fate and behaviour in the environment Study

Data point:	KCA 7.1.1.2/01
Report Author:	Doe, G. E., Roe, T.W.
Report Year:	1986
Report Title:	Anaerobic aquatic metabolism of [pyrimidine-2-^{14}C]SUB A and [phenyl(U)-^{14}C]Sub A in Firebaugh, California, rice paddy sediment.
Report No.:	AMR 606-86
Guidelines followed in study:	USEPA 162-3 (1982)
GLP:	No, GLP was not required at the time this study was conducted. However the work was done in the spirit of GLP.

Executive Summary

The rate and pattern of metabolism of SUB A was studied in non-sterilised and sterilised aquatic rice paddy soil systems from Firebaugh, California, under laboratory anaerobic conditions at 25°C. Individual systems were harvested at 0, 2, 4, 8, 16, 25, 36 and 53 weeks, separated into water and sediment phases, extracted and analysed for SUB A and radiolabelled metabolites.

The first half-life of SUB A was 10-15 weeks in non-sterilised systems and 15-53 weeks in sterilised systems.

In anaerobic aquatic systems treated with ^{14}C-bensulfuron methyl, most of the radiolabel partitioned into the sediment. IN-N5297 (sulfonamide), IN-J0290 (pyrimidine amine), and IN-R9419 were the major degradation products.

Materials and Methods

Test Materials:

Name:	SUB A	
CAS No.:	83055-99-6	
Physical description:	Solid	
Purity:	>99%	>99%
Position of radiolabel:	[phenyl(U)-^{14}C]-SUB A	[pyrimidine-2-^{14}C]-SUB A
Specific activity:	29.8 µCi/mg	16.6 µCi/mg

Table Errore. Nel documento non esiste testo dello stile specificato.**-1. Soil and water characterisation**

Soil name	Firebaugh, California
	Rice paddy sediment
Soil texture*	Silt loam
Sand (%)	25
Silt (%)	63
Clay (%)	12
Organic carbon (%)	1.1
pH	6.5
CEC (meq/100g soil)	17.5
Moisture	saturated
Biomass (mg OC/kg soil) [start/end]	Not measured
	Rice paddy water
pH	7.0

*USDA

Test Procedure

Two radiolabelled forms of SUB A were used: [phenyl(U)-^{14}C]-SUB A (specific activity 29.8 µCi/mg; radiochemical purity >99%) and [pyrimidine-2-^{14}C]-SUB A (specific activity 16.6 µCi/mg; radiochemical purity >99%). Approximately 0.25 mg of each radiolabel was dissolved in 25 mL acetone/water. The rate and pattern of metabolism of SUB A (DPX-F5384) was studied in nonsterilised and sterilised aquatic rice paddy soil systems (Table Errore. Nel documento non esiste testo dello stile specificato.**-1**) under laboratory anaerobic conditions.

Paddy sediment (~2 kg), covered with 3 kg deionised distilled water, was incubated under nitrogen in darkness at 25°C. After 30 days, 75 g wet paddy sediment, equivalent to 48 g dry weight, and 150 mL water were transferred to 250 mL plastic bottles. Sterilised systems were autoclaved at 100°C for 1 hour on three consecutive days. Systems were treated with 0.54 mL 0.0091 mg/mL [phenyl(U)-^{14}C] or 0.62 mL 0.0074 mg/mL [pyrimidine-2-^{14}C]SUB A stock solutions for a nominal rate of 0.1 ppm (0.099 ppm [phenyl(U)-^{14}C]; 0.092 ppm [pyrimidine-2-^{14}C]) based upon the dry weight of the sediment. This rate corresponds approximately to an application rate of 75 g/ha. Test vessels were flushed with nitrogen, tightly capped, and incubated for an additional 53 weeks (non-sterilised) or 36 weeks (sterilised) at 25°C. No attempts were made to trap volatiles.

Water was separated from the soil by filtration and aliquots were analysed by liquid scintillation counting (LSC). Sediment samples were extracted at least four times with 100 mL methylene chloride:methanol:2 M ammonium carbonate (4:3:1; v:v:v). After 4 weeks, samples were also extracted two times by refluxing with 200 mL acetone: 0.1 M ammonium carbonate (9:1, v:v). Non-sterilised 36- and 53-week sediment samples were also refluxed with 0.1 M aqueous NaOH. Extracts were combined and aliquots were analysed by LSC to determine total extractable radioactivity. Extracted soil samples were combusted to determine levels of NER residues.

Samples were concentrated, purified on C18 Bond Elut columns, and analysed by TLC. Degradation products were identified by co-chromatography using authentic standards by TLC.

Results and Discussion

Validity criteria

Material balance (Table Errore. Nel documento non esiste testo dello stile specificato.**-2**) for non-sterilised systems treated with the [^{14}C-phenyl] label ranged from 101.2% of AR at Week 0 to 48.9% of AR at Week 53. Material balance for non-sterilised systems treated with the [^{14}C-pyrimidine] label ranged from 96.3% at Week 0 to 69.0% at Week 53. Material balance for sterilised systems was greater than 94%.

Results of the test

Distribution between water and sediment: For both labels, there was a movement of radioactivity from the water to the sediment (Table Errore. Nel documento non esiste testo dello stile specificato.**-2**). By the end of the study (53 weeks), radioactivity associated with the water fell to approximately 10% or less.

Non-extractable residues: Radioactivity associated with the sediment became more difficult to extract over time so 36- and 53-week sediments were refluxed with 0.1 M NaOH. Non-extractable residues (NER) in 36- and 53-week sediments accounted for a maximum of 7.2% of the applied radioactivity in both labels (Table Errore. Nel documento non esiste testo dello stile specificato.**-2**).

Principal degradation products: SUB A degraded more quickly in non-sterile compared to sterile anaerobic aquatic systems (Table Errore. Nel documento non esiste testo dello stile specificato.**-3** to Table Errore. Nel documento non esiste testo dello stile specificato.**-5**).

In non-sterile soils, the amount of SUB A declined to approximately 16-22% applied radioactivity at week 53. In sterile soils, the amount of SUB A declined to approximately 36-66% of applied radioactivity at week 53.

The major degradation products (>10% AR) in the non-sterilised water and sediment were IN-N5297 (sulfonamide) produced from the phenyl label, IN-J0290 (pyrimidine amine) produced from the pyrimidine label, and IN-R9419 produced from both phenyl and pyrimidine labels. A bridge-intact IN-W8956 was detected in the non-sterile system and reached a maximum of 4.9 % AR at week 8 and decreased to less than 0.1% AR at the end of the study (week 53) in Pyrimidine and Phenyl labels respectively. In sterile system, IN-W8956 reached a maximum of 11.4% AR at Week 36. IN-B6895 (homosaccharin) also reached a maximum of 14.8% in the sterile total system at Week 36. IN-B6895 formation is believed to be an artefact of the considerably more basic soil extraction conditions used in this study.

Table Errore. Nel documento non esiste testo dello stile specificato.**-2. Percent distribution of applied radioactivity and mass balance in anaerobic aquatic systems treated with 0.1 ppm SUB A**

Week	% of Applied Radioactivity			
	Water	Sediment Extractable	Non-Extractable Residues	Total Recovered
Pyrimidine-2-^{14}C (Non-sterile)				
0	70.3	24.0	2.0	96.3
2	39.2	44.6	14.6	98.4
4	28.8	70.6	5.6	105.0
8	22.5	55.1	9.5	87.1
16	21.5	44.9	23.5	89.9
25	21.9	61.8	24.0	107.7
36	10.4	74.5	7.1	92.0
53	10.1	51.7	7.2	69.0
Pyrimidine-2-^{14}C (Sterile)				
0[a]	70.3	24.0	2.0	96.3
2	42.5	51.4	8.8	102.7
16	25.8	57.6	11.5	94.9
36	17.7	66.9	15.7	100.3
Phenyl(U)-^{14}C (Non-sterile)				
0	74.4	24.6	2.2	101.2
2	40.5	50.6	10.7	101.8
4	30.6	62.9	6.1	99.6
8	28.9	53.8	9.5	92.2
16	21.5	30.8	25.3	77.6
25	8.0	52.2	17.2	77.4
36	10.1	44.0	6.1	60.2
53	6.0	37.5	5.4	48.9
Phenyl(U)-^{14}C (Sterile)				
0[a]	74.4	24.6	2.2	101.2
2	52.9	40.4	11.9	105.2
16	61.1	35.2	7.0	103.3
36	59.3	32.4	9.4	101.1

[a] Week 0 is the same as non-sterilised Week 0 sample.

Table Errore. Nel documento non esiste testo dello stile specificato.**-3. Percent distribution of applied radioactivity in the water phase of anaerobic aquatic systems treated with 0.1 ppm SUB A**

Week	% Applied Radioactivity					
	Sub A	**IN-J0290**	**IN-W8956[d]**	**IN-R9419**	**Polars[a]**	**Others[b]**
Pyrimidine-2-^{14}C (Non-sterile)						
0	67.7	<0.1	<0.1	<0.1	<0.1	2.6
2	35.0	1.9	<0.1	<0.1	<0.1	2.3
4	28.1	0.5	<0.1	0.1	<0.1	0.1
8	21.1	0.9	<0.1	0.2	<0.1	0.3
16	1.8	0.3	<0.1	0.8	18.1	0.5
36	2.2	0.9	<0.1	0.8	2.7	3.9
53	2.1	0.7	0.5	1.6	5.2	0.1
Pyrimidine-2-^{14}C (Sterile)						
0[c]	67.7	<0.1	<0.1	<0.1	<0.1	2.6
2	33.5	6.2	<0.1	2.3	<0.1	0.6
16	13.9	4.3	1.0	0.5	5.9	0.2
36	9.0	6.8	<0.1	<0.1	1.2	0.7

Week	**Sub A**	**Sulfonamide**	**IN-J0290**	**IN-W8956[d]**	**Homo-Saccharin**	**Polars[a]**	**Others[b]**
Phenyl(U)-^{14}C (Non-sterile)							
0	74.3	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
2	38.4	1.6	<0.1	<0.1	<0.1	<0.1	0.4
4	23.9	3.9	<0.1	1.0	<0.1	<0.1	1.8
8	15.4	9.9	1.3	1.4	0.9	<0.1	<0.1
16	3.2	3.3	2.5	2.0	3.3	6.7	0.2
36	0.7	6.5	0.1	<0.1	2.8	<0.1	<0.1
53	0.6	0.2	<0.1	0.6	2.0	2.5	<0.1
Phenyl(U)-^{14}C (Sterile)							
0[c]	74.3	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
2	41.6	8.3	<0.1	<0.1	<0.1	<0.1	3.0
16	10.4	7.4	9.7	3.0	7.3	22.2	1.2
36	6.2	22.7	11.4	0.8	14.8	3.5	0.1

[a] Includes origin material and other minor (<10% applied radioactivity) polar unknowns.
[b] Includes background radioactivity in chromatograms.
[c] Sterilised Week 0 is the same as non-sterilised Week 0.
[d] IN-W8956 is Methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]-4-hydroxybenzoate

Table Errore. Nel documento non esiste testo dello stile specificato.**-4. Percent distribution of applied radioactivity in the sediment phase of anaerobic aquatic systems treated with 0.1 ppm SUB A**

Week	% Applied Radioactivity					
	Sub A	**IN-J0290**	**IN-W8956[d]**	**IN-R9419**	**Polars[a]**	**Others[b]**
Pyrimidine-2-^{14}C (Non-sterile)						
0	23.9	<0.1	<0.1	<0.1	<0.1	0.2
2	36.5	3.7	1.4	<0.1	<0.1	3.1
4	46.0	7.6	2.1	10.2	0.1	4.5
8	35.3	5.2	2.5	2.8	1.7	7.6
16	29.1	5.9	3.2	1.7	3.5	1.6
25	39.7	18.2	0.4	2.0	0.4	1.0
36	32.7	4.6	2.3	3.2	26.4[c]	5.5
53	19.4	7.8	<0.1	<0.1	23.0[c]	1.5
Pyrimidine-2-^{14}C (Sterile)						
0[d]	23.9	<0.1	<0.1	<0.1	<0.1	0.2
2	43.0	4.8	<0.1	<0.1	<0.1	3.6
16	49.5	7.8	<0.1	<0.1	<0.1	0.4
36	57.4	6.9	<0.1	<0.1	<0.1	2.5

Phenyl(U)-^{14}C (Non-sterile)							
	Sub A	**Sulfonamide**	**IN-J0290**	**IN-W8956[d]**	**Homo-Saccharin**	**Polars[a]**	**Others[b]**
0	24.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.4
2	47.7	0.8	<0.1	<0.1	<0.1	<0.1	2.1
4	53.1	2.6	1.7	<0.1	2.2	<0.1	3.3
8	36.7	6.0	3.6	2.1	3.2	0.1	2.1
16	23.6	2.1	2.0	1.6	1.6	<0.1	<0.1
25	29.8	8.5	2.7	3.3	2.6	1.4	4.1
36	16.0	4.2	<0.1	14.7	<0.1	5.7	3.2
53	15.5	3.3	<0.1	<0.1	<0.1	18.1[c]	0.6
Phenyl(U)-^{14}C (Sterile)							
0[d]	24.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.4
2	28.9	5.2	<0.1	<0.1	<0.1	<0.1	6.2
16	33.1	<0.1	<0.1	0.9	<0.1	<0.1	1.1
36	29.4	<0.1	<0.1	<0.1	<0.1	<0.1	3.0

[a] Includes origin material and other minor (<10% applied radioactivity) polar unknowns.
[b] Includes background radioactivity in chromatograms.
[c] Includes radioactivity in NaOH reflux extract
[d] Sterilised Week 0 is the same as non-sterilised Week 0.
[d] IN-W8956 is Methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]-4-hydroxybenzoate

Table Errore. Nel documento non esiste testo dello stile specificato.**-5. Percent distribution of applied radioactivity in the total anaerobic aquatic systems treated with 0.1 ppm SUB A**

Week	% Applied Radioactivity					
	SUB A	**IN-J0290**	**IN-W8956[d]**	**IN-R9419**	**Polars[a]**	**Others[b]**
Pyrimidine-2-^{14}C (Non-sterile)						
0	91.6	<0.1	<0.1	<0.1	<0.1	2.8
2	71.5	5.6	1.4	<0.1	<0.1	5.4
4	74.1	8.1	2.1	10.3	0.1	4.6
8	56.4	6.1	2.5	3.0	1.7	7.9
16	30.9	6.2	3.2	2.5	21.6	2.1
25	39.7	18.2	0.4	2.0	0.4	1.0
36	34.9	5.5	2.3	4.0	29.1	9.4
53	21.5	8.5	0.5	1.6	28.2	1.6
Pyrimidine-2-^{14}C (Sterile)						
0[c]	91.6	<0.1	<0.1	<0.1	<0.1	2.8
2	76.5	11.0	<0.1	2.3	<0.1	4.2
16	63.4	12.1	1.0	0.5	5.9	0.6
36	66.4	13.7	<0.1	<0.1	1.2	3.2

Phenyl(U)-^{14}C (Non-sterile)							
	SUB A	**Sulfonamide**	**IN-J0290**	**IN-W8956[d]**	**Homo-Saccharin**	**Polars[a]**	**Others[b]**
0	98.4	<0.1	<0.1	<0.1	<0.1	<0.1	0.5
2	86.1	2.4	<0.1	<0.1	<0.1	<0.1	2.5
4	77.0	6.5	1.7	1.0	2.2	<0.1	5.1
8	52.1	15.9	4.9	3.5	4.1	0.1	2.1
16	26.8	5.4	4.5	3.6	4.9	6.7	0.2
25	29.8	8.5	2.7	3.3	2.6	1.4	4.1
36	16.7	10.7	0.1	14.7	2.8	5.7	3.2
53	16.1	3.5	<0.1	0.6	2.0	20.6	0.6
Phenyl(U)-^{14}C (Sterile)							
0[c]	98.4	<0.1	<0.1	<0.1	<0.1	<0.1	0.5
2	70.6	13.5	<0.1	<0.1	<0.1	<0.1	9.2
16	43.5	7.4	9.7	3.9	7.3	22.2	2.3
36	35.5	22.7	11.4	0.8	14.8	3.5	3.1

[a] Includes origin material and other minor (<10% applied radioactivity) polar unknowns.
[b] Includes background radioactivity in chromatograms.
[c] Sterilised Week 0 is the same as nonsterilised Week 0.
[d] IN-W8956 is Methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]-4-hydroxybenzoate

Calculation of Transformation Rates

The half-life values of SUB A were estimated based on a plot of the percent pyrimidine and phenyl-labelled SUB A vs. time for both the non-sterilised and the sterilised systems. The first half-life of SUB A was 10-15 weeks in non-sterilised systems and 15-53 weeks in sterilised systems.

Conclusions

In anaerobic aquatic systems treated with ^{14}C- SUB A, most of the radiolabel partitioned into the sediment. SUB A degrades mainly *via* hydrolysis of the sulfonylurea bridge to form IN-N5297 and IN-J0290. An alternative pathway involves ester hydrolysis of SUB A to form IN-R9419.

Comments:

PROVA SCRITTA 3

Ecotoxicology Study

Report:	**CA 8.2.6.2/01. Doe, J.M. 1990b**
Title:	**YYY - Toxicity to the Freshwater Diatom (*Navicula Pelliculosa*)**
Report no.:	**90-3-3254**
Guidelines:	**US EPA FIFRA Guideline 122-2 and 123-2.**
Deviations:	**The method used differs from the recommended method (Directive 92/69/EEC Method C3) in the following respect:** **1) The test duration was 120 hours as opposed to 72 hours, which the test guidelines recommend.** **The deviations outlined do not compromise the scientific validity of this study as endpoint relative to 72h exposure can be retrieved.**
GLP:	**Yes**

Materials and methods:

Test material: YYY technical; Batch No. 01; Purity: 98 ± 2%. Measured test concentrations of 0.0063, 0.012, 0.029, 0.053, 0.11 and 0.24 mg/L of YYY technical were introduced into each test flask. A solvent control (acetone) and a dilution water control were set up. The solvent control contained a concentration of acetone which equalled the solvent level in the highest treatment level (0.05 mL). Three replicates at each test concentration were set up. The culture medium used was Algal Assay Procedure (AAP) medium prepared with deionized water and adjusted to pH 7.5±1 with 0.1 NHCl after autoclaving. The stock cultures were maintained under test conditions (shaking rate of 100 rpm, Temperature of 24±2°C, continuous illumination at the surface of the medium of 4.800 lux). The AAP medium used to prepare the exposure solution was formulated in the same manner as the culture medium (excluding Na_2EDTA). Thirty minutes after the test solutions were added to the test flasks, an inoculum of *Navicula pelliculosa* cells calculated to provide 0.3×10^4 cells/mL was aseptically introduced into each flask. The inoculum volume was 890 µL per flask. Reduction in cell density relative to control was measured at 72, 96 and 120 hours after exposure.

Findings:

Analytical results:

Analytical measurements are reported in the table below:

Table CA9. 1: Measured concentrations of YYY used in the 120-hr toxicity test with *Navicula pelliculosa*

Nominal Concentration (mg/L)	Measured Concentrations					
	0-HR		120-HR		MEAN	
	mg/L	%Nom	mg/L	%Nom	mg/L	%Nom
Control	<0.0016	-	<0.0021	-	-	-
Solvent	<0.0016	-	<0.0021	-	-	-
0.0075	0.0065	87%	0.0061	81%	0.0063	84%
0.016	0.0122	77%	0.0108	67%	0.0115	72%
0.033	0.0292	88%	0.0279	85%	0.0285	87%
0.063	0.0574	91%	0.0484	77%	0.0529	84%
0.13	0.1173	90%	0.1102	85%	0.1138	88%
0.25	0.2468	99%	0.264	91%	0.2366	95%
Mean		89%		81%		85%

Biological results:

Cell densities increased over time at all concentrations ≤ 0.053 mg/L. Control densities averaged 68×10^4 cells/ml at 120 hours. Solvent control densities averaged 81×10^4 cells/mL at 120 hours. Solvent control cultures were not significantly different from control cultures at 72, 96 or 120 hours. Data from controls and solvent controls were therefore pooled for EC calculations. Cell densities determined at each observation time are presented in the following tables.

Table CA9.2: Cell density (x 10^4 cells/mL) of *Navicula pelliculosa* after 24 and 48 hours exposure to YYY

Mean Measured Concentration	24 hours				48 hours			
	A	B	C	Mean	A	B	C	Mean
Control	1.00	0.50	0.25	0.58	1.00	3.25	1.75	2.00
Solvent	0.25	0.00	0.25	0.17	1.25	1.75	0.75	1.25
0.0063 mg/L	0.00	0.00	0.25	0.08	2.25	0.75	1.50	1.50
0.012 mg/L	0.00	0.00	0.00	0.00	3.00	0.50	1.25	1.58
0.029 mg/L	0.00	0.00	0.00	0.00	0.00	1.25	0.00	0.42
0.053 mg/L	0.00	0.00	0.00	0.00	1.50	0.75	0.25	0.83
0.11 mg/L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.24 mg/L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table CA9.3: Cell density (x 10^4 cells/mL) of *Navicula pelliculosa* after 72 and 96 hours exposure to YYY

Mean Measured Concentration	72 hours[a]				96 hours[a]			
	A	B	C	Mean	A	B	C	Mean
Control	12.25	19.25	10.25	13.92	29.75	51.75	30.00	37.17
Solvent	11.00	9.50	22.00	14.17	40.50	54.75	36.75	44.00
0.0063 mg/L	10.50	9.25	17.00	12.25	26.00	27.25	38.00	30.42
0.012 mg/L	8.00	9.50	12.00	9.83	22.75	25.75	35.75	28.08
0.029 mg/L	6.50	7.00	9.25	7.58	9.75	13.00	13.50	12.08
0.053 mg/L	3.25	3.75	2.50	3.17	4.25	3.00	3.25	3.50
0.11 mg/L	0.75	0.50	1.25	0.83	1.50	1.25	0.75	1.17
0.24 mg/L	0.50	0.25	0.25	0.33	0.00	0.75	0.50	0.42

[a] Cells were sonicated before counting to break up clumps

Table CA9.4: Cell density (× 10^4 cells/mL) of *Navicula pelliculosa* after 120 hours exposure to YYY

Mean Measured Concentration	120 hours[a]			
	A	B	C	Mean
Control	59.00	75.25	69.00	67.75
Solvent	65.00	78.75	100.50	81.42
0.0063 mg/L	67.25	69.50	90.25	75.67
0.012 mg/L	60.00	49.25	49.75	53.00
0.029 mg/L	13.75	13.00	16.75	14.50
0.053 mg/L	5.50	4.00	5.25	4.92
0.11 mg/L	1.00	0.50	0.75	0.75
0.24 mg/L	1.50	0.00	0.75	0.75

[a] Cells were sonicated before counting to break up clumps

Generally, calculated EC values decreased over the course of the exposure period. No visible abnormalities were observed in any of the cultures. Clumping of cells, such as occurred in all cultures, is normal for this species of diatom. Results of EC_{10}, EC_{50} and EC_{90} calculations for 72, 96 and 120 hour observation intervals are presented in the following table.

Table CA9. 5: EC_{10}, EC_{50} and EC_{90} Values for YYY Calculated from Results of the 120 Hour Toxicity Test with Diatom *Navicula pelliculosa*

Time (hours)	EC_{10} (mg/L)	EC_{50} (mg/L)	EC_{90} (mg/L)
72	0.0062	0.025	0.098
96	0.0062	0.018	0.060
120	0.0087	0.019	0.045

Validity criteria:

Not all validity criteria of the current OECD guideline No. 201 (2006) were met. The mean coefficient of variation for the section-by-section specific growth in the control cultures was 76.1% and 93.1% in the solvent control, which exceeded 35% in both occasions. Due to the type of algae tested, this variation occurs often and this would be due to the clumping of the navicular cells.
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was less than 7% (2.2% and 3.9% for control and S.control respectively), which meets the validity criteria and the biomass in the control cultures have increased exponentially by a factor of at least 16 within the test period (271 and 226 for control and S.control respectively).

Conclusion:

The 72 hour EC_{50} of YYY to *Navicula pelliculosa* was 0.025 mg/L with 95% confidence limits of 0.0076 – 0.079 mg/L. The 120 hour EC_{50} of YYY to *Navicula pelliculosa* was 0.019 mg/L with 95% confidence limits of 0.0094 – 0.038 mg/L.

Comments:

PROVA SCRITTA 3

Mammalian toxicology Study

Compound X (purity 93.4%) was administered to five groups of 7-week-old Wistar rats (12 rats of each sex per group) at a dietary concentration of 0, 800, 4000, 10 000 or 20 000 ppm (equal to 0, 54, 283, 743 and 1545 mg/kg bw per day for males and 0, 62, 320, 788 and 1886 mg/kg bw per day for females, respectively) for 90 days. Achieved concentrations and homogeneity were verified by analysis. All animals were checked for overt clinical signs by daily general cage-side observations. Detailed clinical observations were performed once weekly during the study. Ophthalmological examination was performed on all animals in the control (0 ppm) and high-dose (20 000 ppm) groups in week 12. Body weights and feed consumption were measured weekly during the study. Functional observational battery (FOB) tests were performed weekly on all animals during the study; motor activity assessment was performed in week 12. Blood samples were taken after fasting at termination of the study for assessment of blood biochemical and haematological parameters. Urine analyses were conducted in the first six numbered males and females in week 12. All animals were subjected to detailed postmortem gross examination, and all abnormalities were recorded. Organ weights (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes and epididymides, thymus and uterus) were recorded. Histological evaluation was performed on an extensive list of organs and tissues.

One female from the 4000 ppm group died. There were no treatment-related clinical signs during the study. There was no treatment-related effect on feed consumption at any dose. Reduced body weight (9% for males and 4% for females) and body weight gain (15% for males and 11% for females) were recorded at the end of treatment for both sexes given 20 000 ppm. No treatment-related differences between the controls and any treated group were noted in urine analysis, motor activity or ophthalmological examination. Forelimb grip strength was statistically significantly reduced in male animals in the highest-dose group. Some haematological parameters attained statistical significance at the highest dose.

Key body weight, FOB, clinical chemistry and haematological findings of the 90-day dietary toxicity study in rats

	Males						Females					
Finding	**0 ppm**	**800 ppm**	**4 000 ppm**	**10 000 ppm**	**20 000 ppm**	**HCR[a] or DR**	**0 ppm**	**800 ppm**	**4 000 ppm**	**10 000 ppm**	**20 000 ppm**	**HCR[a] or DR**
Body weight (g)												
Week 0	195.3	193.3	188.2	189.9	189.6	–	150.5	145.7	149.7	146.2	149.3	–
Week 13	383.4	397.4	381.2	373.8	349.9	DR**	233.7	225.0	225.6	231.6	223.5	–
Body weight gain (g)												
Weeks 0–13	188.1	204.1	193.0	183.9	160.2	DR**	83.1	79.3	78.6	85.5	74.2	

Finding	Males						Females					
	0 ppm	800 ppm	4 000 ppm	10 000 ppm	20 000 ppm	HCR[a] or DR	0 ppm	800 ppm	4 000 ppm	10 000 ppm	20 000 ppm	HCR[a] or DR
Functional observational battery												
Mean forelimb grip strength (kg)	1.023	1.008	1.052	1.070	0.760*	–	0.751	0.787	0.707	0.757	0.742	–
SD	(0.270)	(0.253)	(0.292)	(0.149)	(0.235)	–	(0.207)	(0.178)	(0.205)	(0.264)	(0.233)	–
Mean hindlimb grip strength (kg)	0.613	0.642	0.625	0.732	0.665	–	0.620	0.612	0.607	0.620	0.633	–
SD	(0.126)	(0.191)	(0.178)	(0.132)	(0.118)	–	(0.124)	(0.157)	(0.161)	(0.117)	(0.373)	–
Haematology												
Hb (g/dL)	16.7	16.5	16.5	16.6	16.2**	14.5–18.0	15.7	15.7	15.3	15.7	15.0*	14.2–17.0
MCH (pg)	18.2	18.1	18.0	17.9	17.5*	16.6–19.8	18.8	18.4	18.6	18.8	18.4	–
MCHC (g/dL)	35.5	35.4	34.9	35.1	34.6*	31.5–36.2	34.9	34.3	34.5	34.0	34.2	–
Neutrophils (10^9/L)	1.3	1.0	1.1	1.2	0.9**	0.5–3.0	0.6	0.5	0.6	0.6	0.6	–
Monocytes (10^9/L)	0.2	0.2	0.2	0.1	0.1*	0.0–0.3	0.1	0.1	0.1	0.1	0.1	–
Clinical chemistry												
AST (IU/L)	75	65*	66	69	62**	50–87	68	65	64	62	56**	49–103
ALT (IU/L)	46	33*	32*	39	32*	26–69	29	29	32	30	28	–
GGT (IU/L)	2	2	2	2	5**	–	2	2	2	3	4**	–
Na (mmol/L)	145	144*	144	144*	145	132–148	144	143	143	143	144	–
K (mmol/L)	4.7	4.8	4.7	4.7	4.9	–	4.1	4.1	4.1	4.7**	4.7**	3.5–5.0
Ca (mmol/L)	2.71	2.67	2.66	2.71	2.74	–	2.73	2.71	2.71	2.85**	2.88***	2.54–2.91
Total protein (g/L)	69	70	70	71	71	–	71	70	70	74	77**	63–78
Albumin (g/L)	45	45	46	47	47	DR**	48	49	47	50	51*	34–56

Finding	Males						Females					
	0 ppm	800 ppm	4 000 ppm	10 000 ppm	20 000 ppm	HCR[a] or DR	0 ppm	800 ppm	4 000 ppm	10 000 ppm	20 000 ppm	HCR[a] or DR
Total bilirubin (µmol/L)	2.1	2.0	1.5*	1.4*	1.4**	0.5–2.9	2.1	1.8	1.4**	2.0	2.2	1.0–3.5
Total cholesterol (mmol/L)	2.0	2.2	2.3	2.6**	2.8***	–	1.5	1.7	1.8	2.5***	2.6***	–
Creatinine (µmol/L)	37	35	35	36	36	–	40	40	38	40	34**	26–51

ALT: alanine aminotransferase; AST: aspartate aminotransferase; DR: significant dose–response test; GGT: gamma-glutamyl transferase; Hb: haemoglobin; HCR: historical control range; IU: international units; MCH: mean cell haemoglobin; MCHC: mean cell haemoglobin concentration; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; SD: standard deviation.

[a] Historical control data from studies performed between March 2005 and January 2008. For haematology and clinical chemistry, 15 different studies were available (203 and 223 animals).

Total cholesterol was increased (up to 73%) compared with controls, and the increase was statistically significant in both sexes receiving 10 000 and 20 000 ppm. Gamma-glutamyl transferase (GGT) activity was increased by 2–2.5 times compared with controls, and the increase was statistically significant in both sexes given 20 000 ppm. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were statistically significantly decreased in some male treated groups (approximately 18% or less). A statistically significant decrease in AST activity (18%) was noted in 20 000 ppm females.

Organ weight effects were reported only in the liver, for which increased absolute and/or relative weights were recorded in males of the 4000, 10 000 and 20 000 ppm groups and females of the 10 000 and 20 000 ppm groups. Mottled liver was also noted in males given 4000, 10 000 and 20 000 ppm. These changes were accompanied by hepatocyte hypertrophy in both sexes at 4000, 10 000 and 20 000 ppm. Incidence and severity increased with dose and correlated with large and/or mottled liver. Hepatocyte hypertrophy was characterized by hepatocytes with increased levels of pale, slightly granular cytoplasm. In the most severely affected livers, hypertrophy was recorded in hepatocytes in all zones of the liver. In less severely affected livers, there was no clear zonal distribution of the enlarged hepatocytes. Agonal congestion/haemorrhage was recorded for some treated males and was considered to be a consequence of the liver hypertrophy by the study author.

Key macroscopic and microscopic findings of the 90-day dietary toxicity study in rats

Finding	Males					Females				
	0 ppm	800 ppm	4 000 ppm	10 000 ppm	20 000 ppm	0 ppm	800 ppm	4 000 ppm	10 000 ppm	20 000 ppm
Body weight (g)	370.0	381.5	364.5	354.1	339.2	227.1	219.8	220.6	22.9	218.5
Absolute liver weight (g)	8.36	9.03	9.50***	10.18***	12.02***	5.80	5.53	6.08	6.79	8.34***
% change	–	*7.9*	*13.6*	*21.8*	*43.8*	–	*−4.7*	*4.8*	*17.0*	*43.8*

Finding	Males					Females				
	0 ppm	800 ppm	4 000 ppm	10 000 ppm	20 000 ppm	0 ppm	800 ppm	4 000 ppm	10 000 ppm	20 000 ppm
Relative liver weight (%)	2.26	2.37	2.61***	2.89***	3.54***	2.55	2.51	2.76	3.05***	3.81***
% change	*–*	*4.9*	*15.5*	*27.9*	*56.6*	*–*	*−1.6*	*8.2*	*19.6*	*49.4*
Macroscopic pathology: liver										
No. examined	*12*	*12*	*12*	*12*	*12*	*12*	*12*	*11*	*12*	*12*
Large	0	0	2	7	10	0	0	0	0	4
Mottled	0	0	1	3	5	0	0	0	0	0
Microscopic pathology: liver										
No. examined	*12*	*12*	*12*	*12*	*12*	*12*	*12*	*11*	*12*	*12*
Hepatocyte hypertrophy[a]										
Grade -	12	12	0	0	0	12	12	7	3	0
Grade 1	0	0	5	0	0	0	0	4	6	0
Grade 2	0	0	7	0	0	0	0	0	3	0
Grade 3	0	0	0	7	0	0	0	0	0	0
Grade 4	0	0	0	5	1	0	0	0	0	3
Grade 5	0	0	0	0	11	0	0	0	0	9
Agonal congestion/haemorrhage										
P[b]	0	0	1	3	5	2	0	0	0	0
Microscopic pathology: thyroid										
Follicular cell hypertrophy[a]										
No. examined	*12*	*12*	*12*	*12*	*12*	*12*	*12*	*11*	*12*	*12*
Grade -	10	10	6	3	5	10	11	7	7	6
Grade 1	2	2	6	8	5	2	1	4	5	6
Grade 2	0	0	0	1	2	0	0	0	0	0
Microscopic pathology: kidney										
Hyaline droplets[a]										
No. examined	*12*	*12*	*12*	*12*	*12*	*12*	*0*	*0*	*0*	*12*
Grade -	2	3	1	1	0	12	0	0	0	12
Grade 1	6	6	6	3	2	0	0	0	0	0
Grade 2	4	3	4	7	8	0	0	0	0	0
Grade 3	0	0	1	1	2	0	0	0	0	0
Kidney α_{2u}-globulin-positive reaction by immunohistochemistry[a]										
No. examined	*2*	*0*	*0*	*0*	*2*	*0*	*0*	*0*	*0*	*0*
Grade -	0	0	0	0	0	0	0	0	0	0

Finding	Males					Females				
	0 ppm	**800 ppm**	**4 000 ppm**	**10 000 ppm**	**20 000 ppm**	**0 ppm**	**800 ppm**	**4 000 ppm**	**10 000 ppm**	**20 000 ppm**
Grade 1	1	0	0	0	0	0	0	0	0	0
Grade 2	1	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	2	0	0	0	0	0

ppm: parts per million; ***: $P < 0.001$ (analysis of variance, dose–response and Dunnett's, Kruskal-Wallis, Jonckheere-Terpstra or Wilcoxon)

[a] "-": finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe, 5 = severe.

[b] P = finding recorded as present (not graded).

In the thyroid, an increased incidence of follicular cell hypertrophy was noted in animals given 4000, 10 000 and 20 000 ppm. The incidence was higher and the grade was more severe in the males. Follicular cell hypertrophy was characterized by a diffuse increase in the height of the follicular epithelium, generally with a minor reduction in the level of colloid present.

In the kidney, the severity of hyaline droplets was higher in males given 10 000 and 20 000 ppm; the lesion was characterized as densely eosinophilic, variably sized droplets in the cytoplasm of proximal tubular cells. Immunohistochemistry was performed on two control males and two males given 20 000 ppm, and all were positive for α_{2u}-globulin. The positive intensity for the α_{2u}-globulin was higher in the two males given 20 000 ppm, when compared with the controls.

Conclusion:

PROVA SCRITTA 3

Methods of Analysis Study

Method validation for the determination of ACTIVE SUBSTANCE X residues in plant matrices

Report	Determination of ACTIVE SUBSTANCE X residues at harvest in raw agricultural commodity **maize** following a single application in the furrow at ridging of the formulated product
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method consisted in an extraction by Soxhlet with acetone. The extract containing the active ingredient was cleaned up by liquid-liquid partition and the fractions obtained were collected and concentrated to dryness by vacuum rotatory evaporator, and re-dissolved with cyclohexane. The samples were further clean up on silica gel column, the purified extract was dried, re-dissolved with cyclohexane and then analysed by a gas chromatograph equipped with a µ-ECD detector.

The specimens were homogenised by vegetable grinder in order to obtain a homogenous sample. If the analyses were not performed immediately after homogenization, the homogenised samples were stored at -18°C, in a tightly-closed glass or plastic flask.

The LOQ was set at 0.01 mg/kg for plant samples and 0.02 mg/kg for grain samples.

Results and discussions

Table A 1: Recovery results from method validation of ACTIVE SUBSTANCE X using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 2)	Mean recovery (%)	RSD (%)
Maize (plant)	ACTIVE SUBSTANCE X	0.01	95.69	17.9
Maize (plant)	ACTIVE SUBSTANCE X	0.1		

Matrix	**Analyte**	**Fortification level (mg/kg) (*n* = 2)**	**Mean recovery (%)**	**RSD (%)**
Maize (grain)	ACTIVE SUBSTANCE X	0.02	90.37%	3.2%
Maize (grain)	ACTIVE SUBSTANCE X	0.2		

Table A 2: Characteristics for the analytical method used for validation of ACTIVE SUBSTANCE X residues in maize

	ACTIVE SUBSTANCE X
Calibration (type, number of data points)	ACTIVE SUBSTANCE X by GC-ECD 0.01 µg/mL to 2 µg/mL (4 concentrations in solvent standards). Slope = 6.15 x 10^{-8}, intercept = -0.00021 Correlation coefficient (r): 0.9905
Assessment of matrix effects is presented	NO
Limit of determination/quantification	LOQ: Plant samples: 0.01 mg/kg Grain samples: 0.02 mg/kg

Conclusion:

PROVA SCRITTA 3

Residues Study

Determination of Y and Y1 residues in/on rice after one application in four trials - 2 sites in italy and 2 sites in spain (2014)

Summary

The objective of the study is to determine the magnitude of residues of Y and its metabolite Y1 in the raw agricultural commodity rice (paddy grain and straw) following one application of the product XYZ, a SC formulation containing 400 g/L of X (nominal concentration).
Four residue trials were conducted in rice during 2014 in Southern Europe (2 in Italy and 2 in Spain). XYZ was applied once at a target rate of 750 mL/ha (= 300 g X/ha) at crop stage ranging from BBCH 12 to 21. Specimens of rice from the untreated and treated plots were sampled at normal commercial harvest. The specimens were frozen on the same date of collection in the field at -18°C until delivery to the Laboratory.
Residues are extracted with acetonitrile (twice). Two aliquots were taken from the extraction solution. One aliquot for the analysis of Y is evaporated to dryness, taken up in acetonitrile : water (50:50, v/v) and analysed by HPLC-MS/MS. The second aliquot for the determination of Y1 is cleaned up by solid phase extraction using Oasis Max cartridges, evaporated to dryness, taken up in acetonitrile : water (50:50, v/v) and analysed by HPLC-MS/MS.
The limit of quantification (LOQ) was 0.01 mg/kg for Y and Y1, respectively, in all matrices (rice grain, straw, whole plant). The limit of detection (LOD) for Y and Y1, respectively, was set at 0.003 mg/kg in all matrices (rice grain, straw, whole plant).

Residues of Y in rice are summarised below. The EU MRL for rice is 0.01 mg/kg.

Table 1: Proposed use for rice

Crop	NE, SE, GH	Growth Stage	Maximum Number of Applications	Minimum Application Interval [days]	Maximum		Minimum PHI [days]
					Rate [kg a.s./ha]	Water [L/ha]	
Rice	SE	BBCH 00-21 (rice) BBCH 00-12 (target)	1	not relevant	0.20 - 0.3 0	200 - 60 0	not relevant PHI is covered by the vegetation period between application and harvest

SE = Southern Europe, NE = Northern Europe, GH = greenhouse

Trial ID Study ID Report No. Location year	**Crop Variety**	**Method of Treatment**	**No. of Appls**	**Appl Rate (g as/ha)**	**Appl Date**	**Growth stage at Last Appl (BBCH)**	**Portion Analysed**	**PHI (days)**	**Residues of Y (mg/kg)**	**Residues of Y1 (mg/kg)**
1119-1122HSAG13/r 1119.H.SAG13/r Olcenengo (VC) Italy 2013	Rice gladio	Foliar broadcast appl. Boom sprayer	1	296.2	3 july 2013	21 34 83-85 89	whole plant whole plant whole plant whole plant grain (paddy rice) straw	0 30 61 90 90 90	1,4 0,03 <LOQ nd nd nd	0,17 <LOQ nd nd nd nd
			1	596.7	3 july 2013	21 34 83-85 89	whole plant whole plant whole plant whole plant grain (paddy rice) straw	0 30 61 90 90 90	4,4 0,3 0,02 0,03 <LOQ 0,06	0,36 0,02 nd nd nd <LOQ
1119-1122HSAG13/r 1120.H.SAG13/r Borgo Vercelli (VC) Italy 2013	Rice volano	Foliar broadcast appl. Boom sprayer	1	328.3	24 june 2013	21	grain (paddy rice) straw	93 93	nd 0,09	nd 0,01
			1	573.3	24 june 2013	21	grain (paddy rice) straw	93 93	nd 0,16	nd 0,02
1119-1122HSAG13/r 1121.H.SAG13/r Borgo Vercelli (VC) Italy 2013	Rice centauro	Foliar broadcast appl. Boom sprayer	1	310	24 june 2013	19-21	grain (paddy rice) straw	93 93	nd nd	nd <LOQ
			1	576.7	24 june 2013	19-21	grain (paddy rice) straw	93 93	nd <LOQ	nd <LOQ
1119-1122HSAG13/r 1122.H.SAG13/r La Puebla del Río (Seville) Spain 2013	Rice puntal	Foliar broadcast appl. Boom sprayer	1	324	3 july 2013	21	grain (paddy rice) straw	118 118	nd nd	nd nd
				616	3 july 2013	21	grain (paddy rice) straw	118 118	nd nd	nd nd
1113-1116HSAG14/r 1113.H.SAG14/r Olcenengo (VC) Italy 2014	Rice cristallino	Foliar broadcast appl. Boom sprayer	1	288.4	19 june 2014	21	grain (paddy rice) straw	120 120	nd nd	nd nd
1113-1116HSAG14/r 1114.H.SAG14/r Borgo Vercelli (VC) Italy 2014	Rice loto	Foliar broadcast appl. Boom sprayer	1	278.4	19 june 2014	21	grain (paddy rice) straw	120 120	nd <LOQ	nd nd
1113-1116HSAG14/r 1115.H.SAG14/r Dos hermanas (Seville) Spain 2014	Rice puntal	Foliar broadcast appl. Boom sprayer	1	317.6	11 july2014	12	grain (paddy rice) straw	112 112	nd nd	nd nd

1113- 1116HSAG14/r 1116.H.SAG14/r La puebla del rio (Seville) Spain 2014	Rice Mare CL	Foliar broadcast appl. Boom spryer	1	312.7	21 july2014	21	grain (paddy rice) straw	101 101	nd nd	nd nd

Conclusion: