

BIOCHEMISTRY DEPARTMENT
AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
GREENSBORO, NC

UPTAKE AND CHARACTERIZATION OF ϕ -¹⁴C-CGA-48988 AND
ITS SOIL METABOLITES IN FIELD ROTATION SUGAR BEETS

M6-69-5PR, 5SR

Report No.: ABR-79005 Issued By: 5.1.2.e Woo
Submitted By: 5.1.2.e Woo Issue Date: February 12, 1979

A B S T R A C T

Sugar beets were grown as a rotation crop to white potatoes in a field plot on the CIBA-GEIGY Research Farm at Livingston, New York. The plot was treated by spraying ϕ -¹⁴C-CGA-48988 over-the-top six times at a rate of 0.40 lb. a.i./A and at fourteen day intervals. Chemical names, codes and structures are in Figure 1. The first spraying was 45 weeks prior to planting the sugar beets.

The level of radioactivity in the 0-3" soil layer decreased from approximately 0.33 ppm at the time of planting to 0.20 ppm twenty weeks later. During the twenty week growing season for sugar beets, the extraction and partition data show a decrease in organic soluble radioactivity from 36.5% to 11.1% and an increase in nonextractable radioactivity from 48.7% to 80.4%. Since no accumulation of radioactivity occurred in the polar fraction (<10%), nonpolar compounds, possibly parent ϕ -¹⁴C-CGA-48988, were being adsorbed to soil particles. The level of radioactivity remained constant at approximately 0.17 (6-9"), showing that ϕ -¹⁴C-CGA-48988 and its soil metabolites do not leach. Therefore, ϕ -¹⁴C-CGA-48988 and its soil metabolites are probably being degraded to ¹⁴CO₂ slowly.

The uptake of soil radioactivity by whole rotation sugar beets was 0.16 ppm equivalent to ϕ - ^{14}C -CGA-48988 after six weeks of growth. The radioactivity decreased to 0.06 and 0.03 ppm for tops and roots after fifteen weeks and to 0.02 ppm for both tops and roots after twenty weeks (maturity). The extraction and partition data for the six week old sugar beets indicate that as much as half of the recovered radioactivity was in the polar fraction (53.1% of recovered ^{14}C). These data also show that metabolism is from nonpolar to nonextractable products.

Dit document is auteursrechtelijk beschermd en valt onder de verplichting van het Ctgb en wordt beschikbaar gemaakt op grond van de Wet van 1912 betreffende de openbaarmaking van de inhoud van dit document, verspreiding, vermenigvuldiging, omtrent gegevensbescherming inbreuk opleveren van de rechten van derden rusten, waaronder intellectuele rechten van derden rusten, of de inhoud hiervan zonder de toestemming van de rechthebbende van dit document, kan derhalve verboden zijn en een inbreuk opleveren van de rechten van deze rechthebbende.

This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements.

The document may be subject to rights of third parties.

Furthermore, this document may fall under a regulatory data protection regime. Consequently, any publication, distribution, reproduction and/or publishing and any commercial exploitation and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the right of its owner.

INTRODUCTION

N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester*, CGA-48988, is a fungicide proposed for the control of late blight in potatoes. The objectives of this study were to: 1) determine the uptake of the soil degradation products of [U-ring- ^{14}C] N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester** in rotation sugar beets, 2) determine the movement and degradation of ϕ - ^{14}C -CGA-48988 in field soil and 3) characterize the radioactive metabolites of ϕ - ^{14}C -CGA-48988 in soil and sugar beets grown as a rotation crop to potatoes (1).

EXPERIMENTAL

Preparation and Planting of the Field Plot: A 3' x 19' plot was prepared and planted with white potatoes at the CIBA-GEIGY Research Farm at Livingston, New York. The potatoes were sprayed over-the-top six times at 14 day intervals with ϕ - ^{14}C -CGA-48988 at a rate of 0.40 lb. a.i./A. The first spray treatment was six weeks after plant emergence (7/1/77). The ϕ - ^{14}C -CGA-48988 was dissolved in an ethanol/water (1:1) solution and applied with a miniature boom sprayer. Forty-five weeks after the first treatment, a 3' x 3' subplot was prepared for rotation sugar beets by tilling to a depth of approximately 8". Seeds were planted at three inch intervals in three rows spaced twelve inches apart.

Radioactive Dose: A total of 1,755 mg of ϕ - ^{14}C -CGA-48988 was applied to the plot (sp. act. = 30 $\mu\text{Ci}/\text{mg}$ or 9.01 mCi/mM). No additional CGA-48988 or radioactive chemicals were applied for the duration of the studies.

Sampling: Planting and sampling dates are given in Table I. Monthly rainfall data are in Table II. Sugar beets were sampled at 6, 9, 15, and 20 weeks after planting. Soil was sampled at planting and at each sugar beet sampling. Soil cores were divided into 0-3", 3-6" and 6-9" segments for analysis.

*Chemical names and structures are given in Figure 1.

**Hereafter referred to as ϕ - ^{14}C -CGA-48988.

ABR-79005
Page 4 of 12
February 12, 1979

Sample Preparation and Analysis: Plants were homogenized with dry ice in a Wiley Mill (2) and 150-200 mg samples were combusted in a Harvey Oxidizer (3). Biphasic extractions were in accordance with AG-214 to produce organic, polar and nonextractable fractions (4).

Soil samples of approximately two grams each were combusted in a Harvey Oxidizer (3). Extractions were in accordance with AG-254 (5).

Samples were analyzed upon arrival.

Radioactivity Measurements: Radioassays were done in a Beckman LS-255 or Searle Mark III liquid scintillation counter. Efficiencies were obtained by external standardization. Limits of detection and quantitation were determined in accordance with AG-276 (6).

RESULTS AND DISCUSSION

Soil: The levels of radioactivity equivalent to ϕ - ^{14}C -CGA-48988 in field soil (Table V) are shown in Table III. The level in the 0-3" and 3-6" layers showed a drop from approximately 0.33 ppm at the time of planting to 0.20 ppm twenty weeks later. The radioactivity in the 6-9" remained constant at approximately 0.17 ppm throughout the study. These data indicate that the radioactivity associated with the ϕ - ^{14}C -CGA-48988 and its metabolites did not leach. The radioactive decrease may be due to slow degradation to $^{14}\text{CO}_2$ based on prior data (7).

The balance data show a trend of decreasing radioactivity in the organic fraction from 36.5% to 11.1% accompanied by an increase in the nonextractable fraction from 48.7% to 80.4%. There was no accumulation in the polar fraction which remained near the level of quantitation and never exceeded 10% of the total. These data indicate that nonpolar radioactive materials, possibly some parent ϕ - ^{14}C -CGA-48988, were adsorbed to soil particles and are not extractable with methanol/water.

169

ABR-79005
Page 5 of 12
February 12, 1979

Sugar beets: The levels of radioactivity in rotation sugar beets equivalent to ϕ - ^{14}C -CGA-48988 are shown in Table IV. The radioactivity at all samplings and in all plant parts is 0.16 ppm or less. Mature roots and tops contain 0.02 ppm. There was only sufficient radioactivity in the 6 week sample for a balance determination. The recovery does show that half of the radioactivity is in the polar fraction. The recovery of radioactivity in the organic (33.8%) and nonextractable (11.4%) fractions shows that metabolism proceeds through polar to nonpolar and finally to nonextractable products.

These data show that rotation sugar beets take up very little CGA-48988 or its metabolites from soil.

This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements. rights of third parties. Furthermore, any publication, distribution, reproduction and/or publishing and any commercial exploitation and use of this document may therefore be prohibited and violate the right of its owner.

Dit document is eigendom van het Ctgb en wordt beschikbaar gemaakt onder de verplichting tot openbaarmaking, waaronder intellectuele eigendomsrechten, of de inhoud hiervan zonder de toestemming van de rechthebbende, vermenigvuldiging, commercieel gebruik en/of verspreiden van dit document, kan derhalve verboden zijn en een schending van de rechten van de rechthebbende.

ABR-79005
Page 6 of 12
February 12, 1979

REFERENCES

1. [redacted] 5.1.2.e Woo [redacted], ABR-78013, "Uptake of ϕ - ^{14}C -CGA-48988 in Potatoes Grown in a Field Plot - Preparation of Rotational Plots."
2. [redacted] 5.1.2.e Woo [redacted], AG-223, "Blending of Soils and Homogenization of Biological Materials for Radioassay and Extraction."
3. [redacted] 5.1.2.e Woo [redacted], AG-252, "Radioassay of ^{14}C in Biological Materials Using the Harvey Biological Material Oxidizer (BMO)."
4. [redacted] 5.1.2.e Woo [redacted], AG-214, "Biphasic Extraction of Radioactive Metabolites from Treated Biological Material."
5. [redacted] 5.1.2.e Woo [redacted], AG-254, "Extraction of CGA-10832 Residues in Soil."
6. [redacted] 5.1.2.e Woo [redacted], AG-276, "Statistical Methods in the Measurement of Radioactivity."
7. [redacted] 5.1.2.e Woo [redacted], Project Report 08/78, "Degradation of CGA-48988 (Ridomil®) in Soil under Aerobic, Aerobic/Anaerobic and Sterile/Aerobic Conditions."

TABLE I: PLANTING AND SAMPLING DATES

<u>Date</u>	<u>Elapsed Time*</u> (weeks)	<u>Action</u>
5/12/78	0 (45)	Sugar beets planted
6/27/78	6 (51)	25% sample
8/7/78	9 (54)	50% sample
9/15/78	15 (60)	75% sample
10/26/78	20 (65)	Mature sample

*Numbers in parentheses indicate elapsed time (weeks) since the first treatment with ϕ -¹⁴C-CGA-48988 (7/1/77). Subsequent treatments were at 14 day intervals: 7/15/77, 7/28/77, 8/11/77, 8/25/77, and 9/8/77.

This document is not the property of the Ctgb and only provided based on the information requirements, reproduction and/or publishing. Commercial exploitation and use of this document may be prohibited and violate the right of its owner.

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijk verlichtingsruimte tot openbaarmaking. Voorts kan dit document kunnen eigendomsrechten van auteursrechten vallen. Publicatie, verspreiding, vermenigvuldigen, rusten, verspreiden en gebruik van dit document, of de inhoud daarvan te kopiëren, te verspreiden, te verspreiden of te verspreiden, is zonder de toestemming van de rechthebbende van de rechthebbende van de rechthebbende inbreuk op de rechten van de rechthebbende.

Furthermore, any publication, distribution, reproduction and/or publishing of this document may be prohibited and violate the right of its owner.

TABLE II: CIBA-GEIGY NEW YORK RESEARCH FARM MONTHLY RAINFALL DATA; MAY-OCTOBER, 1978

<u>Month</u>	<u>Inches of Precipitation</u>
May	4.9
June	4.1
July	3.4
August	5.5
September	2.8
October	2.3

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking. Voorts kan dit document kunnen worden verspreid, verspreiding, vermenigvuldiging, of de inhoud hiervan zonder de toestemming van de rechthebbende van dit document, kan derhalve verboden zijn en een inbreuk opleveren van de rechten van deze rechthebbende.

This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements. Furthermore, this document may be subject to rights of third parties. rights of third parties. distribution, reproduction and/or publishing and any commercial exploitation and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the right of its owner.

TABLE III: DISTRIBUTION AND BALANCE OF RADIOACTIVITY EQUIVALENT TO ϕ - ^{14}C -CGA-48988 IN FIELD SOIL.

Interval (weeks)*	0 (45)			6 (51)			9 (54)			15 (60)			20 (65)		
	0-3	3-6	6-9	0-3	3-6	6-9	0-3	3-6	6-9	0-3	3-6	6-9	0-3	3-6	6-9
Depth (inches)	0-3	3-6	6-9	0-3	3-6	6-9	0-3	3-6	6-9	0-3	3-6	6-9	0-3	3-6	6-9
Total ppm	0.33	0.30	0.19	0.26	0.25	0.19	0.19	0.15	0.13	0.16	0.16	0.16	0.20	0.20	0.18
Balance (% of Total ^{14}C in 0-3" Layer)															
Organic	36.5			30.6			14.1			12.9			11.1	9.8	<*6.7
Polar	9.1			5.0			5.4			<*6.3			5.7	7.4	<*5.3
Nonext.	48.7			61.2			76.7			85.8			80.4	87.5	95.8
Total	94.3			96.8			96.2			98.7			97.2	104.7	95.8

*Numbers in parentheses indicate the elapsed time (weeks) since the first treatment with ϕ - ^{14}C -CGA-48988 (7/1/77).

<*indicates that the level of radioactivity was below the level of quantitation.

This document is not the property of the Ctgb and only provided based on
 the information contained in this document and/or its production and/or
 reproduction and use of this document may be prohibited and liable to
 the owner of its rights of third parties. Distribution, reproduction and
 use of this document may fall under a regulatory protection and/or
 other rights of third parties.

Op dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt
 op grond van een wettelijke verplichting tot openbaarmaking. Het
 gebruik van dit document, verspreiden, kopiëren, verspreiden of anderszins
 openbaar maken van dit document, of de inhoud hiervan verspreiden, kopiëren
 of anderszins openbaar maken, kan derhalve strafbaar zijn en een
 inbreuk opleveren van de rechten van de rechthebbende.

Furthermore, this document is not the property of the Ctgb and only provided based on
 the information contained in this document and/or its production and/or
 reproduction and use of this document may be prohibited and liable to
 the owner of its rights of third parties. Distribution, reproduction and
 use of this document may fall under a regulatory protection and/or
 other rights of third parties.

The document may be subject to rights of third parties. Distribution, reproduction and
 use of this document may fall under a regulatory protection and/or
 other rights of third parties.

TABLE IV: UPTAKE AND BALANCE OF RADIOACTIVITY EQUIVALENT
 TO ϕ - ^{14}C -CGA-48988 IN ROTATION SUGAR BEETS

Interval (weeks)	6		9		15		20	
	Whole plants	Whole plants	Whole plants	Tops	Whole plants	Tops	Whole plants	Tops
Total ppm	0.16	0.07	0.06	0.03	0.02	0.02	0.02	0.02
Balance	(% of Total ^{14}C)							
Organic	33.8							
Polar	53.1							
Nonext.	11.4							
Total	98.3							

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking. Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten van auteursrechten. Voorts kan dit document worden gebruikt op grond van een wettelijke verplichting tot openbaarmaking. Publicatie, verspreiding, vermenigvuldiging, commercieële exploitatie en gebruik van dit document, of de inhoud hiervan zonder de toestemming van de rechthebbende van de rechten van deze rechthebbende, kan derhalve verboden zijn en een inbreuk opleveren van de rechten van deze rechthebbende.

This document is not the property of the Ctgb and only provided based on the mandatory freedom of information requirements. Consequently, rights of third parties, such as intellectual property and/or copyright, may be subject to a regulatory data protection and use of this document may be prohibited. The document may be published, reproduced, distributed, and/or used without the permission of the owner of this document or its contents. Furthermore, any commercial exploitation, reproduction and/or use of this document may be prohibited. The document may be published, reproduced, distributed, and/or used without the permission of the owner of this document or its contents.

TABLE V: CHARACTERISTICS OF FIELD PLOT SOIL

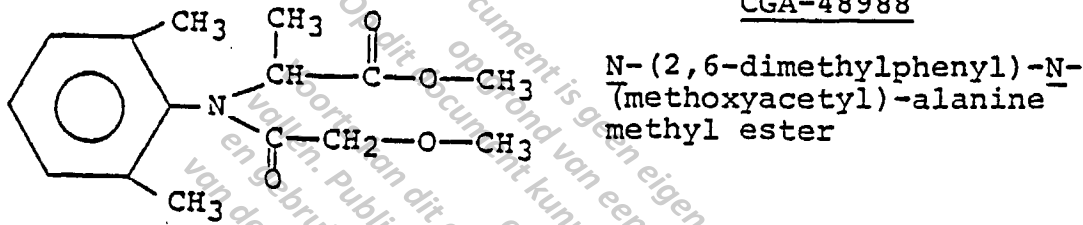
Location: CIBA-GEIGY New York Research Farm,
 Livingston, New York

Texture	Silt	Loam
pH	5.5	
% Organic Matter	1.8	
% Sand	44.4	
% Silt	44.0	
% Clay	11.6	

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking. Voorts kan dit document worden verspreid, vermenigvuldigd, commercieel exploiteren en/of auteursrechten van derden rusten, waaronder intellectuele eigendomsrechten en/of auteursrechten. Het gebruik van dit document, of de inhoud daarvan, is niet de rechthebbende van dit document, maar kan de rechthebbende van de rechten van deze rechthebbende. Dit document kan de rechten van de rechthebbende van deze rechthebbende. Het gebruik van dit document, of de inhoud daarvan, is niet de rechthebbende van dit document, maar kan de rechthebbende van de rechten van deze rechthebbende.

The document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements. Furthermore, this document may be subject to rights of third parties. rights of third parties. Furthermore, any publication, distribution, reproduction and/or publishing without the permission of the owner of this document or its contents may be prohibited and violate the right of its owner. therefore be prohibited and violate the right of its owner.

CGA-48988



Radioactive Compound

ϕ - ^{14}C -CGA-48988

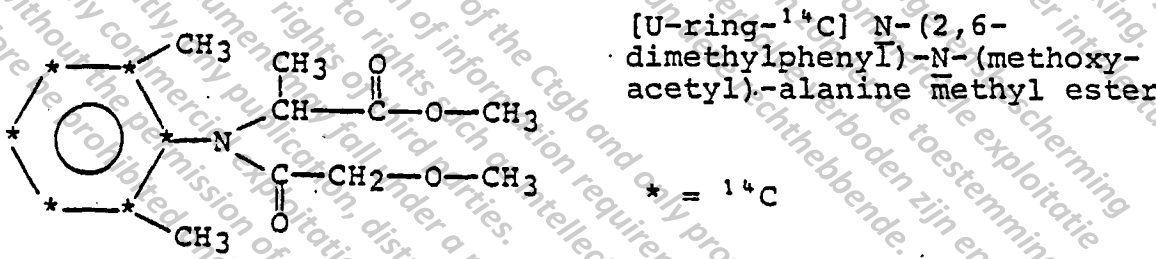
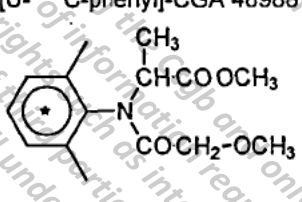


Figure 1: CHEMICAL NAMES AND STRUCTURES

Annex II - 6.2. /08 : Uptake and characterization of Φ - ^{14}C -CGA 48988 and its soil metabolites in field rotation sugar beets

General Information	
Title of the study:	Uptake and characterization of Φ - ^{14}C -CGA 48988 and its soil metabolites in field rotation sugar beets
Report and/or project number:	ABR-79005
Author:	[REDACTED]
Ciba File Number (Desire):	48988/3565
Name and address of testing facility:	Ciba-Geigy Corp., Livingston, NY, USA (Biological phase) Ciba-Geigy Corp., Greensboro, N.C., USA (Analytical phase)
Study period:	5/78 - 10/78
Date of report:	February 12, 1979
Compliance with GLP:	Yes [] No, but complies with sound scientific principles [X]
Test guideline(s) used:	-
Deviations from the test guideline:	-

Test substance	
Test substance (code number):	CGA 48988
Batch:	-
^{14}C -labeled test substance :	Yes [X] No []
Specific activity of [U- ^{14}C -phenyl] label:	1.11 MBq/mg (= 30 μCi /mg)
Radiochemical purity of test substance:	not available
Structural formula: (Position of label)	[U- ^{14}C -phenyl]-CGA 48988 
Formulation used for study:	Yes [] No [X]

Test system	
Target crop:	field grown potatoes
Formulation (spray application): Formulation N° (spray application): Solvent for application (if used):	ethanol/water (1:1) solution
Application: Field experiment:	Spray applications with a miniature boom sprayer: 6 over-the-top sprays (starting 6 weeks after plant emergence) at 14 days intervals at a rate of 0.40 lb./A (= 292.3 mg ^{14}C -CGA 48988 / 3' X 19' plot/ treatment = 8.77 mCi), i.e. 1755 mg ^{14}C -CGA 48988/ 6 treatments (= 52.65 mCi for all 6 applications)
Rotational crop (planting / harvest):	sugar beets: planting: 45 weeks after the first treatment of target potatoes harvest: 20 weeks after planting the soybeans or 65 weeks after the first treatment of target potatoes

Soil:	Soil from Livingston, NY, USA
	Texture: Silt Loam
	pH: 5.5
	% Organic Matter: 1.8
	% Sand: 44.4
	% Silt: 44.0
	% Clay: 11.6

Summary of findings

Sugar beets were grown as a rotation crop to white potatoes in a field plot on the CIBA-GEIGY Research Farm at Livingston, New York. The plot was treated by spraying Φ -¹⁴C-CGA 48988 over-the-top six times at a rate of 0.40 lb. a.i./A and at fourteen-day intervals. The first spraying was 45 weeks prior to planting the sugar beets.

The level of radioactivity in the 0 - 3" soil layer decreased from approximately 0.33 ppm at the time of planting to 0.20 ppm twenty weeks later. During the twenty week growing season for sugar beets, the extraction and partition data show a decrease in organic soluble radioactivity from 36.5% to 11.1% and an increase in nonextractable radioactivity from 48.7% to 80.4%. Since no accumulation of radioactivity occurred in the polar fraction (<10%), nonpolar compounds, possibly parent Φ -¹⁴C-CGA 48988, were being adsorbed to soil particles. The level of radioactivity remained constant at approximately 0.17 ppm (6 - 9"), showing that Φ -¹⁴C-CGA 48988 and its soil metabolites do not leach. Therefore, Φ -¹⁴C-CGA 48988 and its soil metabolites are probably being degraded to ¹⁴CO₂ slowly.

The uptake of soil radioactivity by whole rotation sugar beets was 0.16 ppm equivalent to Φ -¹⁴C-CGA 48988 after six weeks of growth. The radioactivity decreased to 0.06 ppm and 0.03 ppm for tops and roots after fifteen weeks and to 0.02 ppm for both tops and roots after twenty weeks (maturity). The extraction and partition data for the six week old sugar beets indicate that as much as half of the recovered radioactivity was in the polar fraction (53.1% of recovered ¹⁴C). These data also show that metabolism is from nonpolar to nonextractable products.

Tab 1 Uptake, distribution and balance of radioactivity equivalent to Φ -¹⁴C-CGA 48988 in rotation corn and soil (at harvest)

Plant part Soil Layer	Total Residues [ppm]	Organic Phase	Water Phase	Non extractable	Total
Roots	0.02				
Tops	0.02				
0 - 3"	0.20	11.1	5.7	80.4	97.2
3 - 6"	0.20	9.8	7.4	87.5	104.7
6 - 9"	0.18	<*6.7	<*5.3	95.8	95.8

a <* indicates that the level of radioactivity is detectable but below the level of quantitation

PP 2.52/ JK, 11.3.94

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt
 op grond van een wettelijke verplichting tot openbaarmaking.
 Voorts kan dit document eigendomsrechten van derden, waaronder intellectuele
 eigendomsrechten, verspreiding, vermenigvuldiging, commerciële exploitatie
 en gebruik van dit document, of de inbreuk opleveren van deze rechten.
 Op dit document kan derhalve zonder de toestemming
 van de rechthebbende van deze rechten van deze rechten.
 This document is not the property of the Ctgb and only provided based on
 the mandatory freedom of information requirements.
 Consequently, any publication, distribution, reproduction and/or
 rights of third parties.
 Furthermore, any commercial exploitation and use of this document or its
 contents without the permission of the owner of this document may
 therefore be prohibited and violate the right of its owner.