

CLOTHIANIDIN (238) / THIAMETHOXAM (245)

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EXPLANATION

Residue and analytical aspects of clothianidin were considered for the first time by the 2010 JMPR Meeting. The 2010 Meeting estimated the acceptable daily intake (ADI) for humans as 0–0.1 mg/kg bw and estimated the acute reference dose (ARfD) as 0.6 mg/kg bw. The 2010 Meeting defined the residue (for compliance with the MRL and for estimation of dietary intake) for plant and animal commodities as sum of clothianidin and its Z-isomers. The residue is considered not fat soluble.

In 2011 the manufacturer requested JMPR to reconsider the residue definition for clothianidin since the Z-isomer cannot be isolated as such and the Z-isomer should not be included in the residue definition. JMPR agreed to re-evaluate the clothianidin residue definition during the 2011 JMPR meeting. The manufacturer submitted new spectral data to elucidate the equilibrium between clothianidin and its Z-isomer.

IDENTITY

The 2010 JMPR concluded that clothianidin exists predominantly in the E-form. This has been confirmed by NMR analysis [Jeschke *et al.*, 2003]. Quantum chemical calculations revealed that in water the E-isomer is more stable than the Z-isomer. At room temperature the theoretical ratio between E/Z isomers is estimated as 65:1. [Schindler, 2010].

The compound clothianidin is equivalent to the E form of CGA 322704, a metabolite arising from thiamethoxam use. Since thiamethoxam exists as an E/Z mixture, it is likely that the metabolite CGA 322704 exists also as an E/Z mixture. Since insufficient data were available to conclude on the E/Z equilibrium of the CGA 322704 metabolite, the JMPR 2010 included the Z-isomer in the residue definition.

In 2011 the Meeting received additional X-ray diffraction and NMR information as well as quantum chemical calculations to give further information on the E and Z isomers of clothianidin. The E- and Z-forms of clothianidin are indicated in Figure 1.

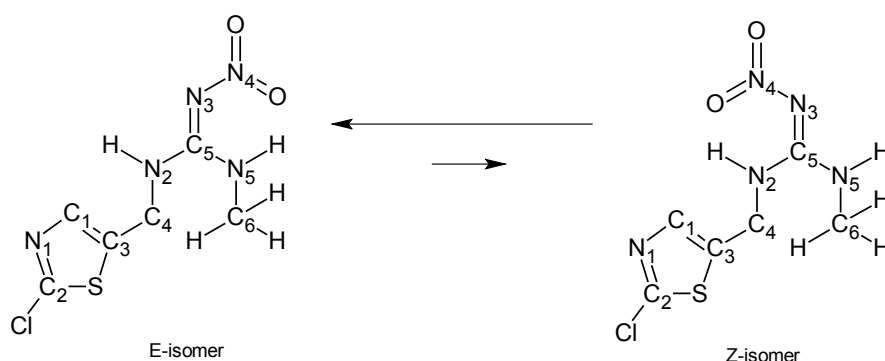


Figure 1 Carbon and nitrogen numbering within the clothianidin molecule (E- and Z-isomer)

Study 1

Jeschke *et al.* (2003) provide an overview of structural information of clothianidin. This study has been taken into account at the 2010 JMPR.

UV VIS spectroscopy

In acidic and neutral solutions a single peak maximum at 265.5 nm is found, while in a basic solution a single peak maximum at 246.0 nm is found. Further details are not indicated.

IR spectroscopy

Clothianidin shows absorption peaks at the following wavelengths: 3330, 3289, 1633, 1542, 1532, 1463, 1433, 1393, 1346, 1320, 1234, 1181, 1141, 1066, 1044 and 949 cm^{-1} . Further details are not indicated.

NMR spectroscopy

NMR spectra of clothianidin were taken by ^1H , ^{15}N , ^{13}C , Heteronuclear Multiple Quantum Correlation (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) NMR. The chemical shifts are referenced to tetramethyl silane (^1H , 0 ppm), deuterodimethyl sulfoxide (^{13}C , 39.7 ppm) or to external nitromethane (^{15}N , 0 ppm). The ^1H , ^{13}C , HMQC and HMBC spectra were recorded from a 0.2 M solution in deuterodimethyl sulfoxide on a 400 MHz spectrometer equipped with a 5 mm quadruple nuclei probe head at +22 °C. The ^{15}N spectrum was recorded from a 0.7 M solution in deuterodimethyl formamide on a 600 MHz spectrometer equipped with a 10 mm selective ^{15}N probe head at +5 °C. NMR signal assignments for clothianidin are indicated in Table 1.

X-ray analysis

Clothianidin crystallizes under normal laboratory conditions as needle-like crystals containing no additional solvent or water molecules. The best quality crystals for X-ray structure analysis were obtained by slow evaporation of a methanol/water (1:1) solution at room temperature. The X-ray reflections up to a torsional angle of 61.1° were measured at –120 °C using a shock cooled crystal under inert oil. X-ray diffraction bond lengths and torsional angles for clothianidin are indicated in Table 2. The 3-dimensional atom coordinates of the analysed molecule are directly derived from these measured X-ray reflections and it confirms that in the crystals clothianidin is exclusively the E-isomer form.

As an additional statement Bayer (2011) indicates that X-ray analysis would have given an additional, clearly different set of X-ray reflections for the Z-isomer of clothianidin if this had been present in the crystal. The existence of significant amounts of the Z-isomer in the crystal can be excluded.

Quantum chemical calculations

Clothianidin was subjected to conformational sampling using force-field methods (MMFF94s) Geometries and free energies were calculated by a DFT/BP/SVP/COSMO-RS model at 298.15 K (25 °C). The optimal geometry for clothianidin as calculated by the DFT/BP/SVP/COSMO-RS model shows a large similarity with the X-ray results (Table 3). For clothianidin the calculated free energies indicate that at room temperature, the preferred orientation of the N-nitroimino group is in the trans position (E-isomer). The Z-isomer (cis position) with lowest energy is more than 10.9 kJ/mol above the optimal E-isomer. The similarity between the X-ray results and the model calculations indicate that clothianidin prefers the same geometry in solution phase as it does in the crystal.

Table 1 NMR signal assignments for clothianidin

	^1H NMR			^{13}C NMR			^{15}N NMR	
Atom	$\delta\text{H/ppm}$	peak shape	rel no H	$\delta\text{C/ppm}$	peak shape	rel no C	$\delta\text{C/ppm}$	peak shape
C1	7.61	S	1	140.3	D	1	–	–
C2	–	–	–	150.9	S	1	–	–

¹ H NMR				¹³ C NMR			¹⁵ N NMR	
Atom	δH/ppm	peak shape	rel no H	δC/ppm	peak shape	rel no C	δC/ppm	peak shape
C3	—	—	—	138.8	S, br	1	—	—
C4	4.51	D, br	2	37.5	T	1	—	—
C5	—	—	—	157.5	S	1	—	—
C6	2.82	D, br	3	28.6	Q	1	—	—
N1	—	—	—	—	—	—	−66.0	S
N2	7.96	br	1	—	—	—	−299.0	D
N3	—	—	—	—	—	—	−143.8	S
N4	—	—	—	—	—	—	−13.9	S
N5	9.18	br	1	—	—	—	−293.6	D

D = doublet, Q = quadruplet, S = singlet, T = Triplet, br = broad

Table 2 X-ray diffraction bond lengths [Å] and torsional angles [°] for clothianidin

Bond	Bond length (angle)	Bond	Bond length (angle)	Bond	Bond length (angle)
S(1)-C(2)	1.7187 (12)	N(2)-C(5)	1.3415 (14)	N(5)-C(6)	1.4506 (15)
S(1)-C(3)	1.7277 (12)	N(2)-C(4)	1.4603 (15)	C(2)-N(1)	1.2909 (16)
N(3)-N(4)	1.3339 (14)	O(2)-N(4)	1.2435 (15)	C(3)-C(1)	1.3605 (17)
N(3)-C(5)	1.3656 (14)	O(1)-N(4)	1.2506 (13)	C(3)-C(4)	1.5001 (17)
Cl(1)-C(2)	1.7111 (13)	N(5)-C(5)	1.3218 (15)	N(1)-C(1)	1.3848 (17)
C(2)-S(1)-C(3)	88.67 (6)	N(5)-C(5)-N(2)	119.27 (10)	O(2)-N(4)-N(3)	124.57 (10)
N(4)-N(3)-C(5)	119.23 (10)	N(5)-C(5)-N(3)	128.56 (11)	O(1)-N(4)-N(3)	114.41 (10)
C(5)-N(2)-C(4)	122.22 (10)	N(2)-C(5)-N(3)	112.16 (10)	N(2)-C(4)-C(3)	113.60 (10)
C(5)-N(5)-C(6)	122.76 (10)	C(1)-C(3)-C(4)	127.21 (11)	C(2)-N(1)-C(1)	108.55 (11)
N(1)-C(2)-Cl(1)	122.85 (10)	C(1)-C(3)-S(1)	108.94 (9)	C(3)-C(1)-N(1)	116.78 (12)
N(1)-C(2)-S(1)	117.04 (10)	C(4)-C(3)-S(1)	123.84 (9)		
Cl(1)-C(2)-S(1)	120.10 (7)	O(2)-N(4)-O(1)	121.02 (11)		

Table 3 Comparison of some key bond lengths [Å] and torsional angles [°] between the DFT/BP/SVP/COSMO-RS model and X-ray diffraction findings

Bond	X-ray-bond length	Model-bond length	Bond	X-ray-angle	Model-angle
N(3)-C(5)	1.3656 (14)	1.36	C(5)-N(2)-C(4)-C3	−78.8	−78.8
N(2)-C(5)	1.3415 (14)	1.35	N(2)-C(4)-C(3)-S1	+60.7	+69.9
N(5)-C(5)	1.3218 (15)	1.34			
N(2)-C(4)	1.4603 (15)	1.46			
N(3)-N(4)	1.3339 (14)	1.35			

Study 2

Schindler (2011a) provided additional quantum chemical calculations. This study report is an updated version of the version taken into account at the 2010 JMPR and now contains information on activation energies.

The optimal geometry for clothianidin was calculated by the DFT/BP/COSMO model. Quantum chemical calculations indicated that in water the E-isomer of clothianidin is more stable than the Z-isomer by approximately 10.5 kJ/mol. At room temperature the theoretical ratio between E/Z isomer is estimated as 65:1. Hence approximately 1.5% of the compound in solution exists in the Z-form at equilibrium. In the gas phase the energy difference is higher (14.6–16.7 kJ/mol) corresponding to a ratio of 750:1 for the E:Z isomer.

Conformational change of the E-isomer to the Z-isomer requires the passage over three transition states on the potential energy surface of the molecule, corresponding to the variation of three torsional angles. The heights of these barriers are calculated to be 58.6, 46.0 and 62.8 kJ/mol, respectively, in water and 54.4, 67.0 and 46.0 kcal/mol in the gas phase. According to the study author, the height of the transition state barriers are comparable to those between the twist-boat and chair forms of cyclohexane (50.2 kJ/mol). Based on the potential energy surface in torsion angle space of clothianidin the experimental determination of another geometric isomer of clothianidin different from the experimentally determined E-isomer at ambient temperature is unlikely.

As an additional statement Bayer (2011) indicates that the calculations of Schindler (2011a) are in line with the NMR measurements of Jank (2011) and show that the E-isomer form of clothianidin is the far preferred form for the molecule. In solution (under physiological conditions, not crystallized) all molecules show vibration and rotation of bonds and they are able to switch from one 3-dimensional form into another if there is enough energy present to “push” them over existing energy barriers that separate the different 3-dimensional forms. As demonstrated by Schindler (2011a), the energy barriers between the different 3-dimensional forms of clothianidin (46.0–62.8 kJ/mol) is low enough to allow spontaneous and rapid interconversion to occur at ambient temperatures. In the E-isomer form clothianidin is more “relaxed” and this is why this form is favoured. This is also the reason why the Z-isomer form of clothianidin will always re-arrange its bonds by rotation until the molecule again can rest in the E-isomer form.

For thiamethoxam itself this is different because thiamethoxam is lacking the hydrogen atoms that can stabilize the nitro group (NO₂) in one or the other position. Consequently, thiamethoxam itself exists as a 1:1 mixture of E- and Z-isomers with no preference. After cleavage of the 6-ring of thiamethoxam and formation of the metabolite CGA 322704 initially there is a 1:1 mixture of E- and Z-isomers of CGA 322704. But this ratio only exists for a very brief moment. Because the Z-isomer of CGA 322704 is the “tensed” form of this molecule and energetically not favoured, and because the energy barrier between the Z- and E-isomers of CGA 322704 is low, it will immediately “relax” to the more favoured E-isomer form and spontaneously build the experimentally proven ratio of dominating E-isomer with virtually no Z-isomer detectable under physiological conditions.

From this theoretical study it is clear that the E-isomer form is the most stable form and that at room temperature clothianidin and the CGA 322704 metabolite of thiamethoxam are likely to exist as an equilibrium of 1.5% Z-isomer and 98.5% E-isomer (E/Z ratio 66:1).

Study 3

Schindler (2011b) provided quantum mechanical calculations of the expected NMR shifts of the E- and Z-isomers of clothianidin. The calculated proton NMR shifts are strongly dependent on the solvent. The quantum mechanical calculations predict drastic changes of the ¹H shifts of the N2 and N5 protons between the E- and Z-isomers (Table 4), which permits an unambiguous identification of the two forms. The ¹³C and ¹⁵N spectra are predicted to be too similar for the two isomers and would not allow such a discrimination.

Table 4 ¹H-NMR signal predictions for clothianidin for the E- and Z-isomer

	E-isomer			Z-isomer		
Atom	δH in gas	δH in ACN	δH in DMSO	δH in gas	δH in ACN	δH in DMSO
C1	7.71	7.62	7.68	7.76	7.95	8.36
C2	–	–	–	–	–	–
C3	–	–	–	–	–	–

	E-isomer			Z-isomer		
Atom	δH in gas	δH in ACN	δH in DMSO	δH in gas	δH in ACN	δH in DMSO
C4	4.20, 5.46	4.38, 5.60	4.38, 5.57	4.77, 4.53	4.53, 4.84	4.38, 5.18
C5	–	–	–	–	–	–
C6	4.35	3.09	3.08	2.99	2.91	2.91
N1	–	–	–	–	–	–
N2	4.35	6.56	7.96	10.84	10.87	10.79
N3	–	–	–	–	–	–
N4	–	–	–	–	–	–
N5	10.01	10.26	10.26	3.97	5.94	7.96

Study 4

Jank (2011) provided NMR spectra at room temperature and at low temperature to reduce or eliminate possible exchange line broadening. Saturated solutions of clothianidin were prepared in deuterated acetonitrile, chloroform and methanol. 1H -NMR spectra were recorded at 298.0 K (25 °C), 273.0, 253.0 and 233.0 K on a 400 MHz spectrometer.

NMR spectrum in deuterated acetonitrile (CD_3CN)

At room temperature (298 K) the proton spectrum showed the expected resonances of the C1, C4 and C6 protons as sharp signals ($\delta H = 7.51, 4.56, 2.86$, respectively). Two broad signals represented the N2 and N5 protons ($\delta H = 6.7\text{--}5.9$ and $9.5\text{--}8.7$, respectively). No signals of any other isomer were detected. Lowering the temperature to 273, 253 and 233 K gradually revealed a sharpening of the N2 and N5 signals and the appearance of a second set of signals that were assigned to a second, minor isomer of clothianidin (Table 5). The assignment was based on the observed scalar splitting patterns and a two dimensional NMR spectrum (1H - 1H -COSY at 233 K).

NMR spectrum in deuterated chloroform ($CDCl_3$)

At room temperature (298 K) the proton spectrum showed only the resonances of the C1, C4 and C6 protons as sharp signals ($\delta H = 7.46, 4.67, 2.97$, respectively). No signals of the N2 and N5 protons were detectable. At 273 K, clothianidin started to precipitate and experiments were stopped.

NMR spectrum in deuterated methanol (CD_3OD)

At room temperature (298 K) the proton spectrum showed the expected resonances of the C1, C4 and C6 protons as sharp signals ($\delta H = 7.54, 4.61, 2.93$, respectively). No signals of the N2 and N5 protons were detectable. Going down in temperature, further signals appeared in the spectrum, none of which could be unequivocally assigned to a second isomer of clothianidin.

Results from the acetonitrile spectrum showed that the E- and Z-isomer of clothianidin were both present in solution. The major E-isomer showed N2 protons as a triplet at $\delta H = 6.47$ and N5 as a quartet at $\delta H = 9.18$. The minor isomer basically showed an inversion of these shifts: the triplet of N2 was now observed at $\delta H = 9.67$ and the quartet of N5 at $\delta H = 5.97$. Re-evaluation of the spectra at 253, 273 and 298 K, allowed the detection of the Z-form at 253 and 273 K, but not at 298 K (25 °C). At 298 K, the Z-isomer could also not be detected in a 600 MHz spectrometer with a cryo probe. Based on the 400 MHz signals at 233 K, the content of the Z-isomer was estimated to be 3.5% (w/w).

The calculated and the measured NMR shift values were in good agreement for both isomers including the two-NH proton signals that differed significantly between the isomers, thus confirming the interpretation of the NMR signals in the low temperature experiments.

The purpose of the low temperature NMR experiment is to increase the lifetime of the unfavoured isomer in its shallow energy minimum. By lowering the temperature it was possible to "freeze" the Z-isomer for a time long enough to be detected by NMR. From the NMR spectra it is clear that in solution clothianidin consists of 3.5% Z-isomer and 96.5% E-isomer (E/Z ratio 27:1) at 233.0 K. These new study results demonstrate that clothianidin predominantly exists in the E-form

and that experimental separation of the minor Z-isomer from the E-isomer is not possible at ambient temperature.

Table 5 ¹H-NMR signal assignments for clothianidin in CD₃CN at 233.0 K for the E- and Z-isomer

E-isomer				Z-isomer		
Atom	δH/ppm	peak shape	rel no H	δH/ppm	peak shape	rel no H
C1	7.50	S	1	7.56	S	1
C2	—	—	—	—	—	—
C3	—	—	—	—	—	—
C4	4.51	D	2	4.55	D	2
C5	—	—	—	—	—	—
C6	2.81	D	3	2.73	D	3
N1	—	—	—	—	—	—
N2	6.47	T	1	9.67	T	1
N3	—	—	—	—	—	—
N4	—	—	—	—	—	—
N5	9.18	Q	1	5.97	Q	1

D = doublet, Q = quadruplet, S = singlet, T = Triplet, br = broad

APPRAISAL

Residue and analytical aspects of clothianidin were considered for the first time by the 2010 JMPR Meeting. The 2010 Meeting established an acceptable daily intake (ADI) of 0–0.1 mg/kg bw per day and estimated the acute reference dose (ARfD) as 0.6 mg/kg bw. The 2010 Meeting defined the residue (for compliance with the MRL and for estimation of dietary intake) for plant and animal commodities as sum of clothianidin and its Z-isomer. The residue is considered not fat soluble.

In 2011 the manufacturer requested JMPR to reconsider the residue definition for clothianidin since the Z-isomer cannot be isolated as such and the Z-isomer should not be included in the residue definition. JMPR agreed to re-evaluate the clothianidin residue definition during the 2011 JMPR meeting. The manufacturer submitted new spectral data to elucidate the equilibrium between clothianidin and its Z-isomer. As the results apply equally to clothianidin and the thiamethoxam metabolite CGA 322704, the Meeting decided to consider the expression of residue definitions for both compounds.

Chemical structure

The Meeting received quantum mechanical calculations, NMR data and X-ray data to elucidate the equilibrium between clothianidin and its Z-isomer.

Quantum mechanical calculations revealed that the E-isomer form is the most stable form and that at room temperature an equilibrium of 1.5% Z-isomer and 98.5% E-isomer (E/Z ratio 66:1) in water is formed. The calculated transition state barriers between the E- and Z-isomer forms (with 10.5 kJ/mol energy difference) corresponding to the variation of three torsional angles are 58.6, 46.0 and 62.8 kJ/mol, respectively. These transition state barriers are so low that the conversion between the E- and Z-isomer forms at room temperature is rapid and an equilibrium is formed rapidly. The E/Z equilibrium with a ratio of E:Z of 66:1 is formed irrespective whether the starting material is an E/Z mixture (thiamethoxam metabolite CGA 322704) or the E-isomer (clothianidin).

In order to verify the theoretical calculations, NMR experiments at low temperatures were performed with a clothianidin solution in deuterated acetonitrile. As the measurement temperature goes down from room temperature to -4 °C, the lifetime of the unfavoured Z-isomer is increased for a time long, enough to be detected by NMR. Based on spectral data it was confirmed that the E-isomer form is the most prominent form in equilibrium. An E/Z equilibrium with an E/Z ratio of 27:1 is formed in a deuterated acetonitrile solution at -40 °C. At ambient temperature there is no chance to isolate the Z-isomer, because it will always immediately transform back to the E-isomer.

Definition of the residue

The compound clothianidin is equivalent to the E form of CGA 322704, a metabolite arising from thiamethoxam use. Thiamethoxam exists as an E/Z mixture and the 2010 JMPR had insufficient data to conclude on the E/Z equilibrium of the CGA 322704 metabolite. The JMPR 2010 included the Z-isomer in the residue definition.

Based on the additionally submitted structural studies, an E/Z equilibrium with a ratio of E:Z of 27:1 at -40 °C is formed irrespective whether the starting material is an E/Z mixture (thiamethoxam metabolite CGA 322704) or the E-isomer (clothianidin). These new study results demonstrate that experimental separation of the minor Z-isomer from the E-isomer is not possible at ambient temperature because the Z-isomer will always immediately transform back to the E-isomer. For this reason, the Z-isomer of CGA 322704 need not be mentioned in the residue definition for either clothianidin or thiamethoxam.

The Meeting recommended the following as revised residue definition for clothianidin:

Definition of the residue for compliance with the MRL or for estimation of the dietary intake for plant commodities: *clothianidin*

Definition of the residue for compliance with the MRL or for estimation of the dietary intake for animal commodities: *clothianidin*.

The Meeting recommended the following as revised residue definition for thiamethoxam:

Definition of the residue for compliance with the MRL for plant and animal commodities: *thiamethoxam*

Definition of the residue for estimation of the dietary intake for plant and animal commodities (except poultry): *thiamethoxam and clothianidin* (considered separately)

Definition of the residue for estimation of the dietary intake for poultry: *sum of thiamethoxam, CGA 265307 and MU3, expressed as thiamethoxam; and clothianidin* (clothianidin to be considered separately from thiamethoxam).

The changes above do not impact on the recommendations for clothianidin and thiamethoxam nor the dietary risk assessments made by the 2010 JMPR.

REFERENCES

Code	Author	Year	Title, Institute & report number, Submitting manufacturer and report code, GLP/Non-GLP. Published/Unpublished
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