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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL USE OF ASCORBIGEN

(57) Abstract: Use of ascorbigen to inhibit, prevent and/or hinder the development of chronic and/or degenerative conditions in a mammal.

WO 2007/112964 A1

Novel use of Ascorbigen

The present invention relates to a novel use of ascorbigen; in particular it relates to the use of ascorbigen to inhibit, prevent and/or hinder the development of chronic and/or
5 degenerative conditions in a mammal.

Ascorbigen is a known indol derivative which can be isolated from cabbage juice or synthetically prepared from ascorbic acid and 3-hydroxymethylindole. By systematic nomenclature, ascorbigen is a mixture of 2-C(1H-indol-3-ylmethyl)- α -L-lyxo-3-
10 hexulofuranosonic acid γ -lactone and 2-C(1H-indol-3-ylmethyl)- α -L-xylo-3-hexulofuranosonic acid γ -lactone. As used herein, the term "ascorbigen" comprises the particular enantiomeres each individually as well as the above mentioned mixture and (stereo-) isomers and mixtures thereof.

15 Many genes which produce substance that are involved in the protection of cells against carcinogens, oxidants, and other toxic chemicals, are under the transcriptional control of a simple DNA regulatory element: the antioxidant response element (ARE). The signaling system leading to ARE activation has been partly elucidated, and nuclear factor erythroid 2-related factor 2 (Nrf2) has been identified as the key transcriptional
20 factor that serves to transmit the inducer signal to ARE (J Biochem Mol Biol. 2004 Mar 31;37(2):139-43.) It is now known that Nrf2, which is normally sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Kept1), dissociates from it upon exposure to ARE-mediated inducers, translocates to the nucleus, complexes with other nuclear factors, and binds to ARE. ARE-regulated gene products include Phase II
25 enzymes such as glutathione S-transferase, NAD(P)H:quinone oxidoreductase 1, UDP-glucuronosyltransferase, gamma-glutamate cysteine ligase, and hemeoxygenase-1. There is strong evidence that increased expression of ARE-regulated genes inhibits cancer development and degenerative processes (Curr Drug Targets CNS Neurol
Disord. 2005 Jun;4(3):267-81). Accordingly, ARE activators, such as oltipraz, anethole
30 dithiolethione, sulforaphane, 6-methylsulphinyhexyl isothiocyanate, curcumin, caffeic acid phenethyl ester, 4'-bromoflavone, etc. are considered to have a potential in (chemo-) preventing and/or hindering the development of cancer, degenerative

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processes and/or age related diseases in a mammal, including neurological decay, Alzheimer, increasing oxidative stress, muscle decay.

Thus, in one aspect, the present invention relates to the use of ascorbigen to inhibit,
5 prevent and/or hinder the development of chronic and/or degenerative conditions in a mammal.

In a further aspect, the invention relates to a method of inhibiting, preventing and/or hindering the development of chronic and/or degenerative conditions in a mammal,
10 which comprises administering to a mammal in need of such treatment an effective amount of ascorbigen. Examples of such chronic and/or degenerative conditions include neurodegenerative diseases such as neurological decay, Alzheimer's disease or cognitive impairment; eye diseases, such as age-related macular degeneration, muscle decay, and muscle fatigue; and conditions associated with bone decay such as
15 osteoporosis.

While for the purpose of the present invention mammals are preferably humans, the invention is not limited to humans but includes other mammals, especially pets such as horses, dogs, cats and/or small animals, e.g. hamsters and/or guinea pigs.
20

For the purposes of the invention, ascorbigen may be administered to, e.g., a human adult (weighing about 70 kg) in an amount of up to about 10 to 1000 mg/day in one or several dosage units or servings. In a particular embodiment of the invention, the dosage for a human adult (weighing about 70 kg) is up to about 500 mg/day, especially
25 up to about 100 to 200 mg/day. If administered in a food or beverage the amount of ascorbigen contained therein is suitably no less than about 50 mg per serving. In another embodiment of the invention such amount is no less than about 100 mg per serving. If ascorbigen is administered as a pharmaceutical formulation such formulation may contain up to about 500 mg per solid dosage unit, e.g. per capsule.

30 The term "serving" as used herein denotes an amount of food and/or beverage normally ingested by a human adult with a meal at a time and may range, e.g., from about 100 g to about 500 g food and/or beverage.

35 The composition according to the present invention can preferably be a nutraceutical composition. The term "nutraceutical" as used herein denotes usefulness in both, the nutritional and pharmaceutical field of application. Thus, "nutraceutical compositions"

according to the present invention can serve as supplements to food, feed and beverages, dietary supplements and as pharmaceutical formulations which may be solid – such as capsules – or liquid – such as solutions or suspensions. It is evident from the foregoing that the term “nutraceutical composition” also comprises food, feed
5 and beverages containing ascorbigen.

A multi-vitamin and mineral supplement may be added to the compositions according to the present invention, e.g. to maintain a good balanced nutrition or to obtain an adequate amount of an essential nutrient missing in some diets. The multi-vitamin and
10 mineral supplement may also be useful for disease prevention and protection against nutritional losses and deficiencies due to lifestyle patterns and common inadequate dietary patterns sometimes observed in diabetes.

The composition according to the present invention can be a food or beverage
15 composition. Beverages can be e.g. sports drinks, energy drinks or other soft drinks, or any other suitable beverage preparation, e.g. yoghurt drinks, hot beverages or soups. In a preferred embodiment of the present invention the beverage is an “instant beverage”, i.e. a beverage which is produced – normally by the consumer themselves – by stirring a powder into a liquid, usually milk or water.

20 By “sports drink” a beverage is meant which is consumed before, during and/or after physical exercise, mainly to hydrate as well as to (re-) store electrolytes, sugar and other nutrients. Sports drinks are usually isotonic, meaning they contain the same proportions of nutrients as found in the human body.

25 Energy drinks are beverages which contain (legal) stimulants, vitamins (especially B vitamins) and/or minerals with the intent to give the user a burst of energy. Common ingredients include caffeine, guarana (caffeine from the Guarana plant) and/or taurine, various forms of ginseng, maltodextrin, inositol, carnitine, creatine, glucuronolactone,
30 coenzyme Q10 and/or ginkgo biloba. Some may contain high levels of sugar, or glucose, whereas others are sweetened completely or partially with a sugar alcohol and/or an artificial sweetener like Ca-cyclamate or Aspartame. Many such beverages are flavored and/or colored.

35 A soft drink is a drink that does not contain alcohol. In general, the term is used only for cold beverages. Hot chocolate, tea, and coffee are not considered soft drinks. The term includes carbonated and non-carbonated drinks, e.g. mineral water or so-called “near

water drinks”, i.e. water-based beverages which have an additional benefit, e.g. a special flavor and/or further (functional) ingredients. One simple example for a “near water drink” is a mixture of water with very little juice.

- 5 If the composition is prepared in form of one of the following food articles it is according to the present invention advantageous if the amount of ascorbigen in the composition is selected from the ranges given in the following table:

Food Category	typical serving size	Preferred amount ascorbigen in beverage or food (most preferred range in brackets)
Beverages (final product) (e.g. soft drinks, juices (fruit and/or vegetable), teas, soups)	200 mL, preferred 3 servings a day	50-1000 mg/L (150-750) 10-200 mg/serving, (30-150) 30-600 mg/day (90-450)
Dairy Products (e.g. milk shakes, yoghurts, yoghurt drinks, ice creams)	150 g	50-1000 mg/kg (165-660) 8-150 mg/serving (25-100)
Sweets and confectionaries (e.g. chocolates, candies, mints, jellies, cookies)	1 to 3 pieces of 5 g each per day	2000 – 40'000 mg/kg (6000-30'000) 10 – 200 mg/piece (30-150) 10-600 mg/day (30-450)
other food items (e.g. breakfast cereals, muesli bars, snacks, pasta sauces)	25 g	400 - 8000 mg/kg (1200-6000) 10 – 200 mg /serving (30-150)

- 10 If the composition is prepared in form of a capsule it is according to the present invention advantageous if the amount of ascorbigen is selected from the ranges given in the following table:

Category	typical dosage	Preferred amount ascorbigen per capsule (most preferred in brackets)
Capsules	1-2 x per day	20 – 600 mg/capsule (50 – 450)

The invention is further illustrated by Figures 1 to 5:

Figure 1 shows the effect of ascorbigen stimulation on NADPH: Quinone oxidoreductase (NADPH:QO1) RNA expression in HepG2 cells. The y-axis shows the fold induction. Data are average out of 3 independent experiments. * $p < 0.05$.

Figure 2 shows the effect of ascorbigen stimulation on NADPH: Quinone oxidoreductase (NADPH:QO1) enzymatic activity in HepG2 cells. The y-axis shows the induction in %. Data are average out of 3 independent experiments. * $p < 0.05$.

Figure 3 shows the effect of ascorbigen against free radical challenge (Paraquat) on HepG2 cells survival. The y-axis shows the survival rate in %. Data are average out of 3 independent experiments.

* $p < 0.05$

○ Control (untreated)

† Paraquat 500 μ M

‡ Paraquat 500 μ M + ascorbigen 100 μ M

Figure 4 shows the effect of ascorbigen supplementation on RNA expression of NADPH: Quinone oxidoreductase 1 (N:QO1) and UDP-Glucuronosyltransferase in rat liver. The y-axis shows the fold induction.

* $p < 0.05$

○ Control (untreated)

▲ N:QO1 (ascorbigen)

■ UDP-Glucuronosyltransferase (ascorbigen)

Figure 5 shows the effect of ascorbigen supplementation on NADPH: Quinone oxidoreductase 1 (N:QO1) and UDP-Glucuronosyltransferase enzymatic activity in rat liver. The y-axis shows the induction rate in %.

* $p < 0.05$

○ Control (untreated)

▲ N:QO1 (ascorbigen)

■ UDP-Glucuronosyltransferase (ascorbigen)

The invention is further illustrated by the following examples.

Examples:

The efficacy of ascorbigen in the selective activation of phase II enzymes *in vitro* and *in vivo* was demonstrated as shown by tests set forth below.

5 In vitro test model

Liver cells HepG2 were treated with physiological amount of ascorbigen in a dose-dependent fashion for 16 hours. Cells were harvested and RNA isolated. The enzyme NAD(P)H:Quinone Oxidoreductase1 (NQO1) was chosen as a representative for the family of phase II enzymes. Quantification of NQO1 transcript was performed by real
10 time PCR TaqMan®. Likewise, cells were harvested after stimulation with ascorbigen and the enzymatic activity of NQO1 was measured.

Results

Compared to control cells (no treatment), cells stimulated with increasing amount of
15 ascorbigen show a dose-dependent increase of NQO1 transcriptional activity (Figure 1). These results are also confirmed at the enzymatic level with a significant increase of NQO1 activity (Figure 2).

In vivo test model

20 Male Wistar rats weighing 200 - 220 g, after 4 days of labor acclimation were randomized into groups of 10 animals. Following treatment groups have been analyzed i) Control animals receiving placebo, ii) Test animals receiving 5mg/rat/day of Ascorbigen. The compound was administrated daily by gavages for 7 consecutive days. Food and water were given ad libitum during the experiment.

25 The activation of phase II enzymes was measured as following:
Livers were collected; RNA and cellular microsomes were fractionated, isolated and stored at -80°C. Transcriptional activation of the phase II enzyme representatives NADPH:Quinone Oxidoreductase 1 and Glutathione-S-Transferase was assessed by quantitative PCR. Enzymatic activity was measured based on appropriate assays.

30

Results

Compared to control animals (placebo), rats daily supplemented with Ascorbigen, showed a significant increase in liver's mRNA expression of NADPH:Quinone Oxidoreductase 1 and Glutathione-S-Transferase (figure 4). These results were
35 subsequently confirmed by appropriate enzymatic assays. Both phase II enzymes showed a significant increase in their enzymatic activities (figure 5).

Pharmaceutical compositions may be prepared by conventional formulation procedures.

Example 1

Soft gelatin capsule

Soft gelatin capsules are prepared by conventional procedures containing as active
5 ingredient 100 mg of ascorbigen per capsule.

Example 2

Hard gelatin capsule

Hard gelatin capsules are prepared by conventional procedures containing as active
10 ingredient 100 mg of ascorbigen per capsule.

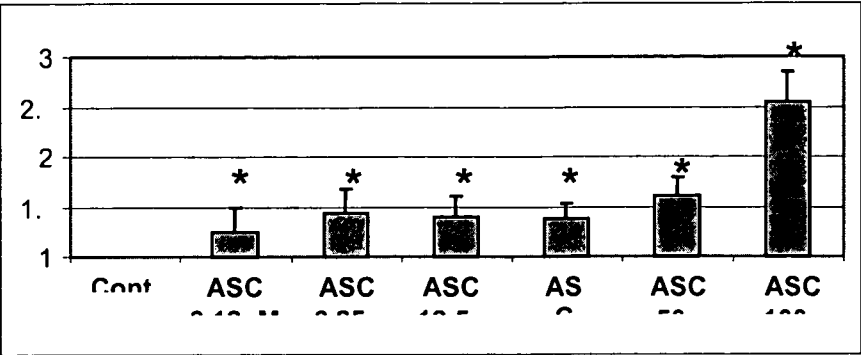
Example 3

Food items may be prepared by conventional procedures containing ascorbigen in an
amount of 50 mg to 500 mg per serving. Examples of such food items are soft drinks,
15 bread, cookies, yoghurt, ice cream, and sweets.

Claims:

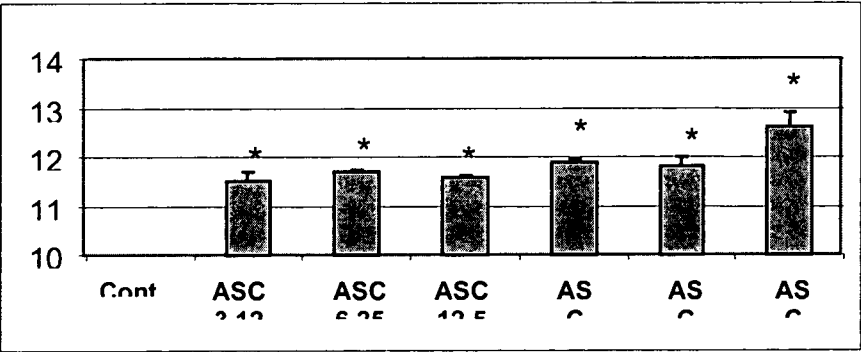
1. Use of ascorbigen to inhibit, prevent and/or hinder the development of chronic and/or degenerative conditions in a mammal.
2. Use according to claim 1 wherein the condition is a neurodegenerative disease.
3. Use according to claim 1 wherein the condition is selected from neurological decay, Alzheimer's disease and/or cognitive impairment.
4. Use according to claim 1 wherein the condition is an eye disease, especially age-related macular degeneration.
5. Use according to claim 1 wherein the condition is muscle decay or muscle fatigue.
6. Use according to claim 1 wherein the condition is associated with bone decay.
7. Use according to claim 6 wherein the condition is osteoporosis.
8. Use of ascorbigen for the manufacture of a composition to inhibit, prevent and/or hinder the development of chronic and/or degenerative conditions in a mammal.
9. Use according to claim 8 wherein the composition is a nutraceutical composition.
10. Use according to claim 8 wherein the composition is a food or beverage or supplement to food or beverage.
11. Use according to any of the claims 9 or 10 wherein the amount of ascorbigen present in the composition is appropriate to provide a dosage of 10 to 1000 mg per day for an adult in one or several dosage units or servings.
12. A method for inhibiting, preventing and/or hindering the development of chronic and/or degenerative conditions in a mammal which comprises administering to a mammal in need of such treatment an effective amount of ascorbigen.
13. The method according to claim 12 wherein the amount of ascorbigen is appropriate to provide a dosage of 10 to 1000 mg per day for an adult.

Figure 1:



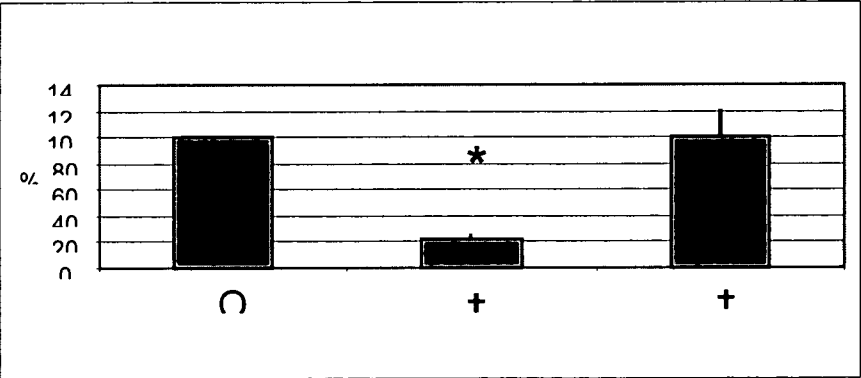
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Figure 2:

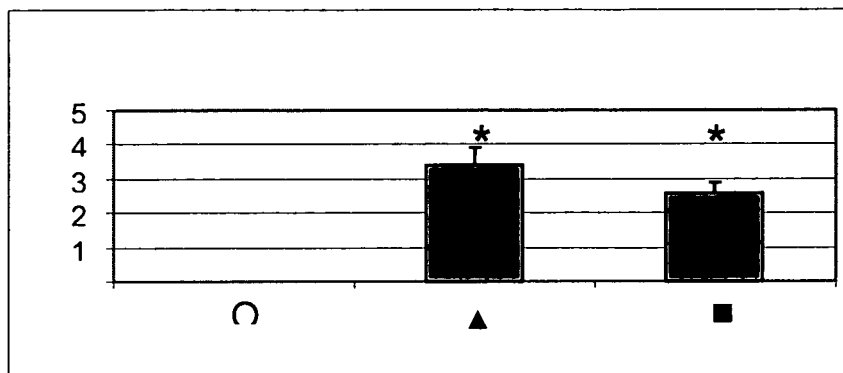


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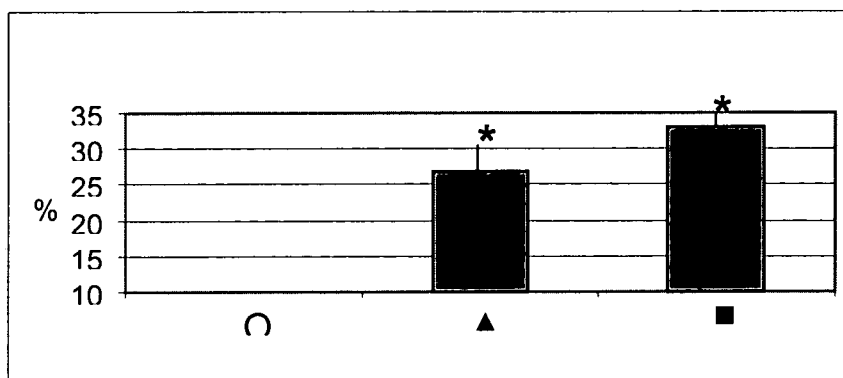
Figure 3:



15

Figure 4:

5

Figure 5:

10

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/002858

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L1/30 A61K36/31 A61K31/404

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE, COMPENDEX, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANONYMOUS: "Verwendung von Ascorbigen als Anti-Aging Mittel" IP.COM JOURNAL, IP.COM INC., WEST HENRIETTA, NY, US, 24 February 2005 (2005-02-24), XP013023450 ISSN: 1533-0001 page 2, line 15 - page 4, line 2 page 7 - page 8	1-13
X	WO 99/17768 A (DESIGNED NUTRITIONAL PRODUCTS [US]) 15 April 1999 (1999-04-15) page 1, line 7 - line 28; claims 1,3,9; example 6 ----- -/-	1,5,8-13

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

8 June 2007

Date of mailing of the international search report

09/07/2007

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/002858

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PREOBRAZHENSKEYA M N ET AL: "Ascorbigen and other indole-derived compounds from Brassica vegetables and their analogs as anticarcinogenic and immunomodulating agents" PHARMACOLOGY AND THERAPEUTICS, vol. 60, no. 2, 1993, pages 301-313, XP002436766 ISSN: 0163-7258 page 308	1,8-13
X	DE 103 08 298 A1 (KULLMER THOMAS [DE]) 9 September 2004 (2004-09-09) the whole document	1,8-13
X	RU 2 235 543 C1 (BUKHMANN V M) 10 September 2004 (2004-09-10) abstract	1,8-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2007/002858

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-7, 12-13
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-7, 12, 13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; It is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2007/002858

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9917768	A	15-04-1999	CA 2306748 A1 EP 1030663 A1 US 5895787 A	15-04-1999 30-08-2000 20-04-1999
DE 10308298	A1	09-09-2004	NONE	
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