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ORIGINAL SUBMISSION

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May 4, 2008

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**Via E-mail and Overnight Mail**

**RECEIVED**  
**MAY 05 2009**

**BY:.....**

Dr. Robert L. Martin  
Office of Food Additive Safety (HFS-255)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
University Station Building  
4300 River Road, Room 2045  
College Park, MD 20740-3835

**Re: GRAS Notification for Arginine When Used in Solution Containing other approved GRAS Substances for Addition to Meats (e.g., Beef and Pork) and Poultry**

Dear Dr. Martin:

Oh behalf of our client, Dr. Ranzel Nickelson, II (the "Notifier"), we submit this letter in triplicate to notify the U.S. Food and Drug Administration ("FDA") of the Notifier's conclusion that the use of arginine as set forth in this document is exempt from the premarket approval requirements applicable to food additives<sup>1</sup> under the Federal Food, Drug, and Cosmetic Act ("FDC Act"), 21 U.S.C. § 301 *et seq.*, because such use is generally recognized as safe ("GRAS"). 21 C.F.R. § 170.30. We also send an additional copy for FDA to forward onto USDA's Food Safety and Inspection Service ("FSIS").

**I. Name and Address of the Notifier**

Ranzell "Nick" Nickelson II, Ph.D.  
455 Sansom Blvd  
Saginaw, TX 76179  
817-916-1332 office  
682-286-0807 fax

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<sup>1</sup> 21 U. S.C. § 348(b)-(f).

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**II. Common or Usual Name of the Substance**

The common or usual name for the substance is "L-Arginine."<sup>2</sup> This document will refer to the substance as "arginine."

**III. Intended Use**

Arginine will be added via a solution as a processing aid to meat and poultry. The solution will contain arginine and other approved GRAS substances. The scope of this petition includes beef subprimals and ground beef, pork, and poultry.

The solution is not re-circulated. It is mechanically injected into the meat/poultry via a machine, which inserts needles into the meat and deposits the solution into the meat. Slides of a sample injection system, Wolf-tec Needle Injector, are attached. See Attachment 2. The solution is injected into meat at levels between 10-20% (16% is current target). Subprimals are injected with a chilled solution to desired pump level. The solution is added to the ground meat at 6-8% prior to forming into patties. For ground beef, the solution is added directly by pouring it into the final blender prior to forming.

**IV. Basis for the GRAS Determination**

The Notifier concludes that arginine is generally recognized as safe for the intended use outlined in this document through scientific procedures.

**V. Availability of Data and Information**

The data and information that are the basis for the GRAS determination are available for CFSAN's review and copying at reasonable time at Dr. Nickelson, 455 Sansom Blvd, Saginaw, TX 76179, or, upon request, can be sent to CFSAN for review.

**VI. Identity, Manufacturing Process, and Specifications of Arginine**

The arginine meets the specifications set forth in the FCC. Suppliers would assure that the arginine meets FCC specifications through a letter of guaranty and certificate of analysis.<sup>3</sup>

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<sup>2</sup> See Food Chemicals Codex, 6<sup>th</sup> Ed. (2008-2009), L-Arginine (Attachment 1).

<sup>3</sup> See Food Chemicals Codex, 6<sup>th</sup> Ed. (2008-2009), L-Arginine.

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The Notifier uses food-grade arginine in a solution, which is comprised of arginine and other approved GRAS substances. Arginine is added at 0.2-0.6% (depending on muscle type and desired pump level).<sup>4</sup> The % values are measured by dry weight. The solution is made under current good manufacturing processes.

**VII. Self-Limiting Levels of Use**

The amount of brine injected (pumped) into subprimals prior to cutting into steaks ranges from 10-20% (current target is 16%). In laboratory evaluations of different levels and concentrations, the Notifier developed a model using fresh ground beef. 50 grams of ground beef was weighed, blended for 15 seconds, and then filtered for 15 minutes. The filtrate is measured and compared to a water control. The Notifier then calculated % Retention Over Control (ROC) to determine optimum levels. The ROC studies demonstrated that there is a law of diminishing returns on the use of arginine. The Notifier also used equivalent levels of Sodium Tripolyphosphate as benchmarks.

As there is some cost associated with arginine, The Notifier has made every effort to insure that minimum levels are used to achieve desired results. Thus, the minimum amount of arginine is added to achieve the intended results.

We previously submitted data to FSIS to support the suitability of the aforementioned intended use. This information on suitability is incorporated by reference in this document.

**VIII. GRAS Determination Through Scientific Procedures**

It is common knowledge that arginine is one of the naturally occurring amino acids in meat and poultry protein.<sup>5</sup> Arginine is reported to be from 5.52 to 6.98% of beef protein,

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<sup>4</sup> For current suitability studies, arginine is used at 0.3%.

<sup>5</sup> The Free Dictionary by Farlex, citing The Columbia Electronic Encyclopedia (2007) (“[O]ne of the 20 amino acids commonly found in animal proteins.”), *available at* <http://encyclopedia2.thefreedictionary.com/arginine> (last visited 5/1/09); The Free Dictionary by Farlex, citing Encyclopedia Concise Britannica (2008) (“One of the essential amino acids, particularly abundant in histones and other proteins associated with nucleic acids. It plays an important metabolic role in the synthesis of urea, the principal form in which mammals excrete nitrogen compounds. Arginine is used in medicine and biochemical research, in pharmaceuticals, and as a dietary supplement.”), *available at* <http://encyclopedia2.thefreedictionary.com/arginine> (last visited 5/1/09); McGraw-Hill Concise Dictionary of Modern Medicine (2002) (“Biochemistry: A 'facultatively' essential

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depending on breed.<sup>6</sup> The total protein content of meat is 16-20% (local data), so the level of arginine in meat is approximately 1.5% or 15,000 ppm.

A study published in *Regulatory Toxicology and Pharmacology* in 2008 assessed the safety of arginine when added to the diet. See Shao, A., Hathcock, J.N., *Risk Assessment for the amino acids taurine, L-glutamine, and L-arginine*, *Regulatory Toxicology and Pharmacology*, 50(2008):376-399 (Attachment 3). The study states that “experts have generally acknowledged the absence of adverse effects from [orally administered] supplemental amounts [of arginine] in humans.” This risk assessment scrutinized data from published human clinical trials, of which there was “a fairly robust human clinical trial dataset.” The study reviewed 38 published human clinical trials and observed, “None of the clinical trials reviewed found any systemic and credible hazard or adverse effects related to [arginine] administration. Therefore, by definition, there is no basis for identifying a NOAEL [no observed adverse effect level] or LOAEL [lowest observed adverse effect level]. In the absence of either of those two values a UL [upper level of intake] usually is not set.” Based on the dosage used in the clinical trials and the quality of the trials, the researchers selected 20 g/d as the OSL (observed safe level) for supplemental arginine. The researchers acknowledged the conservative nature of their exclusive reliance on human data, but stated that the advantage to this approach is that the level identified as safe warrants an extremely high level of confidence.

The intended use of arginine falls well below 20 g/d. The solution is injected into meat at levels between 10-20% (16% is current target). The solution addition level anticipated for ground beef is 6-8%. The level of arginine is 0.2%-0.6% in the solution. Thus, the level of arginine used in the solution represents only approximately 5% of the arginine already present in meat tissue or 600 ppm of finished raw product. In other words,

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amino acid that contains a guanido group with a pKa > 12, which carries a positive charge at physiological pH; it becomes an essential amino acid when the body is under stress or injured. Sources: Turkey, chicken and other meats.”); American Heritage Medical Dictionary (2007) (“An amino acid obtained from the hydrolysis or digestion of plant and animal protein.”).

<sup>6</sup> See Subrt, J., et al. The profile of amino acids in intramuscular protein of bulls of milked and beef commercial types. *Czech J. Anim. Sci.*, 47: 21-29 (2002); see also Uniform Retail Meat Identity Standards (“Beef, bottom sirloin, tri-tip, separable lean only, trimmed to 0” fat, select, raw “shows 3477 mg of arginine per 252 g per serving.”); NutritionData.com, Foods highest in Arginine (list includes beef, pork, lamb, veal, game, poultry, sausages, and luncheon meats).

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the naturally occurring arginine is present at levels more than 20 times greater than that being added by the solution. The naturally occurring arginine and the added arginine would be indistinguishable in laboratory analysis. Exposure through consumption would be 0.06% for the added arginine if the subprimal was injected to the maximum level of 20%. For a 4 ounce serving, there would be 0.068 g of arginine.

As stated earlier, arginine is a naturally occurring component of meat and poultry protein, and the arginine used in the solution is indistinguishable from naturally occurring arginine in the meat/poultry. Any byproducts produced by the addition of arginine to meats/poultry would be consistent with the naturally existing byproducts from arginine present in meat/poultry.

The arginine meets FCC specifications, and the arginine used in the solution is indistinguishable from naturally occurring arginine in the meat/poultry. The arginine is added to the meat/poultry during processing and does not significantly increase the amount of arginine naturally found in the food.

To further support the safety of the intended use described in this document, we note that FDA has classified arginine as an approved food additive under the use conditions defined in 21 C.F.R. § 172.320. The regulation stipulates that the addition of arginine to food is safe under several conditions, one of which is that the amount of the arginine added for nutritive purposes plus the amount naturally present in free and combined (as protein) form must not exceed 6.6% by weight of total protein (expressed as free amino acid) of the finished food.

The addition of arginine as a processing aid in meats and poultry does not introduce any novel safety issues apart from those considered by FDA in its consideration of the arginine use described in 21 C.F.R. § 172.320. The minimum amount of arginine is added to achieve the desired effect, and the amount of arginine added plus the amount naturally present in free and combined form does not exceed 6.6% by weight of total protein (expressed as free amino acid) of the finished food. The added arginine will result in a PER of protein consistent with meat/poultry protein. Moreover, although the arginine is added for a different purpose, it is the same food substance as that provided for in 21 C.F.R. § 172.320 and it is added at or below the levels indicated in 21 C.F.R. § 172.321. Therefore, no novel safety issues arise from the addition of arginine as a processing aid in meats and poultry.

Also, the use of arginine as a flavoring agent has been determined to be safe by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). JECFA states:

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No safety concern at current levels of intake when used as a flavouring agent. Not evaluated using the Procedure for the Safety Evaluation of Flavouring Agents; the substance is a macronutrient and a normal component of protein and, as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as a flavouring agent.

See Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives, Arginine, available at [http://inchem.org/documents/jecfa/jecval/jec\\_156.htm](http://inchem.org/documents/jecfa/jecval/jec_156.htm).

Finally, arginine has been the subject of extensive study as a drug to treat a wide variety of diseases and symptoms (e.g., to measure growth hormone levels in people who might have related deficiencies and to treat people with inborn errors of urea synthesis).<sup>7</sup> It is well recognized in the medical community for its therapeutic uses.<sup>8</sup> Arginine is also widely sold in the U.S. as a dietary supplement, and we have found over two hundred 30-day notification letters submitted to FDA pursuant to FDCA § 403(r)(6) for dietary supplements that contain arginine.<sup>9</sup>

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<sup>7</sup> See MayoClinic.com. Arginine (L-arginine). 2008. [http://www.mayoclinic.com/health/l-arginine/NS\\_patient-arginine](http://www.mayoclinic.com/health/l-arginine/NS_patient-arginine) ("Arginine is considered a semi-essential amino acid because even though the body normally makes enough of it, supplementation is sometimes needed....Early evidence suggests that arginine may help treat medical conditions that improve with vasodilation, such as chest pain, clogged arteries (called atherosclerosis), coronary artery disease, erectile dysfunction, heart failure, intermittent claudication/peripheral vascular disease, and blood vessel swelling that causes headaches (vascular headaches). Arginine also triggers the body to make protein and has been studied for wound healing, bodybuilding, enhancement of sperm production (spermatogenesis), and prevention of wasting in people with critical illnesses."); see also Wikipedia.com. Arginine. <http://en.wikipedia.org/wiki/Arginine> (last visited 4/21/2009).

<sup>8</sup> See Mosby's Dictionary of Complementary and Alternative Medicine (2005) ("[A]n essential amino acid that has been used as an adjunct therapy in congestive heart failure, erectile dysfunction, peripheral vascular disease (PVD), and angina pectoris. It may also be useful in the treatment of upper respiratory ailments, type II diabetes, and various hematologic conditions.").

<sup>9</sup> See e.g., Kohli, R., et al., Dietary L-Arginine Supplementation Enhances Endothelial Nitric Oxide Synthesis in Streptozotocin-Induced Diabetic Rats, The American Society for Nutritional Sciences J. Nutr. 134:600-608. March 2004, available at <http://jn.nutrition.org/cgi/content/abstract/134/3/600>.

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The references above support the conclusion that there is a consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that arginine is safe when added to food as described in this document.

Very truly yours,

Robert G. Hibbert

Attachments

cc: Catherine Rockwell, DAM, FSIS  
Dr. Ranzel Nickelson, II

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Acceptance criterion: NMT 10 mg/kg

### SPECIFIC TESTS

- **RESIDUE ON IGNITION (SULFATED ASH)**, Appendix IIC  
Sample: 2 g  
Acceptance criterion: NMT 0.2%

## Arabinogalactan

Larch Fiber  
Larch Gum

INS: 409

CAS: [9036-66-2]

### DESCRIPTION

Arabinogalactan occurs as a white to yellow-white, coarse or fine powder. It is the dried water extract from the wood of the larch trees *Larix occidentalis* and *Larix laricina* (Fam. Pinaceae). It is a highly branched polysaccharide that has a molecular weight of 15,000 to 60,000 daltons and is composed of galactose units and arabinose units in the approximate ratio of 6 : 1. It is freely dispersible in hot or cold water. It is insoluble in alcohol.

**Function** Dietary fiber; humectant; stabilizer

**Packaging and Storage** Store in well-closed containers.

### IDENTIFICATION

#### A. PROCEDURE

Sample: 20 g

**Sample solution:** Add the *Sample* to 20 mL of water and stir until completely dissolved. Pour the solution into a 500 mL beaker and add 100 mL of water.

**Analysis:** Transfer 7 mL of the *Sample solution* into a 250-mL beaker and add 0.2 mL of diluted lead subacetate TS. [NOTE: Retain the remaining *Sample solution* for the Identification test B (below).]

Acceptance criterion: No precipitate forms.

#### B. PROCEDURE

**Sample solution:** Retained *Sample solution* from Identification test A (above)

**Analysis:** To the *Sample solution*, add 280 mL of 95% ethyl alcohol.

Acceptance criterion: No precipitate forms.

### ASSAY

#### TOTAL CARBOHYDRATES

**Analysis:** Determine the difference between 100% and the sum of the percent *Ash (Total)*, *Loss on Drying*, and *Protein*.

Acceptance criterion: NLT 80% (as arabinogalactan)

### IMPURITIES

#### Inorganic Impurities

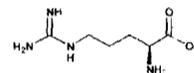
- **LEAD**, *Lead Limit Test*, *Atomic Absorption Spectrophotometric Graphite Furnace*, Method I, Appendix IIIB  
Acceptance criterion: NMT 0.1 mg/kg

### SPECIFIC TESTS

- **ASH (TOTAL)**, Appendix IIC  
Acceptance criterion: 10.0%
- **INSOLUBLE MATTER**  
Sample: 5 g  
**Analysis:** Dissolve the *Sample* in about 100 mL of water contained in a 250-mL Erlenmeyer flask, add 10 mL of 2.7 N hydrochloric acid, and boil gently for 15 min. Filter the hot solution by suction through a tared filtering crucible and wash the residue thoroughly with hot water. Dry the residue at 105° for 2 h, and weigh.  
Acceptance criterion: NMT 0.1%
- **LOSS ON DRYING**, Appendix IIC (105° for 5 h)  
Acceptance criterion: NMT 8%
- **PROTEIN**, *Nitrogen Determination, Method I*, Appendix IIIC  
Sample: 3.5 g  
**Analysis:** Transfer the *Sample* into a 500-mL Kjeldahl flask. Multiply the percentage of nitrogen determined by 6.25.  
Acceptance criterion: NMT 1.0%
- **STARCH**  
Sample solution: 100 mg/mL  
**Analysis:** Add a few drops of iodine TS to the *Sample solution*.  
Acceptance criterion: No blue or red color appears.

## L-Arginine

L-2-Amino-5-guanidinovaleric Acid



C<sub>6</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>

Formula wt 174.20  
CAS: [74-79-3]

### DESCRIPTION

L-Arginine occurs as white crystals or as a white crystalline powder. It is soluble in water, insoluble in ether, and sparingly soluble in alcohol. It is strongly alkaline, and its water solutions absorb carbon dioxide from the air.

**Function** Nutrient

**Packaging and Storage** Store in well-closed, light-resistant containers.

### IDENTIFICATION

#### Change to read:

- **INFRARED ABSORPTION**, *Spectrophotometric Identification Tests*, Appendix IIIC  
Reference standard: USP L-Arginine RS  
Sample and standard preparation: K  
Acceptance criterion: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.▲FCC6

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**ASSAY**• **PROCEDURE**

**Sample:** 200 mg

**Analysis:** Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid to a green endpoint or until the blue color disappears completely. Each mL of 0.1 N perchloric acid consumed in the assay is equivalent to 8.710 mg of  $C_6H_{14}N_4O_2$ .

[CAUTION: Handle perchloric acid in an appropriate fume hood.]

**Acceptance criteria:** NLT 99.5% and NMT 101.5% of  $C_6H_{14}N_4O_2$ , calculated on the dried basis

**IMPURITIES****Inorganic Impurities**• **LEAD, Lead Limit Test, Appendix IIB**

**Sample solution:** Prepare as directed for organic compounds.

**Control:** 5  $\mu$ g Pb (5 mL of *Diluted Standard Lead Solution*)

**Acceptance criterion:** NMT 5 mg/kg

**SPECIFIC TESTS**• **LOSS ON DRYING, Appendix IIC (105° for 3 h)**

**Acceptance criterion:** NMT 1.0%

• **OPTICAL (SPECIFIC) ROTATION, Appendix IIB**

**Sample:** 8 g, previously dried

**Analysis:** Dissolve the *Sample* in sufficient 6 N hydrochloric acid to make 100 mL.

**Acceptance criteria:**

$[\alpha]_D^{20}$  Between +26.0° and +27.9°, on the dried basis; or

$[\alpha]_D^{25}$  Between +25.8° and +27.7°, on the dried basis

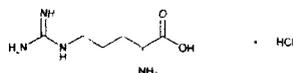
• **RESIDUE ON IGNITION (SULFATED ASH), Appendix IIC**

**Sample:** 1 g

**Acceptance criterion:** NMT 0.2%

**L-Arginine Monohydrochloride**

L-2-Amino-5-guanidinovaleric Acid Monohydrochloride



$C_6H_{14}N_4O_2 \cdot HCl$

Formula wt 210.66

CAS: [1119-34-12]

**DESCRIPTION**

L-Arginine Monohydrochloride occurs as a white or nearly white crystalline powder. It is soluble in water, slightly soluble in hot alcohol, and insoluble in ether. It is acidic and melts with decomposition at about 235°.

**Function** Nutrient

**Packaging and Storage** Store in well-closed, light-resistant containers.

**IDENTIFICATION****Change to read:**• **INFRARED ABSORPTION, Spectrophotometric Identification Tests, Appendix IIC**

**Reference standard:** USP Arginine Hydrochloride RS

**Sample and standard preparation:** K

**Acceptance criterion:** The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*. $\Delta_{FCC6}$

**ASSAY**• **PROCEDURE**

**Sample:** 100 mg, previously dried

**Analysis:** Dissolve the *Sample* in 2 mL of formic acid, add exactly 15.0 mL of 0.1 N perchloric acid, and heat on a water bath for 30 min. [CAUTION: Handle perchloric acid in an appropriate fume hood.] After cooling, add 45 mL of glacial acetic acid, and titrate the excess perchloric acid with 0.1 N sodium acetate, determining the endpoint potentiometrically. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 10.53 mg of  $C_6H_{14}N_4O_2 \cdot HCl$ .

**Acceptance criteria:** NLT 98.5% and NMT 101.5% of  $C_6H_{14}N_4O_2 \cdot HCl$  on the dried basis

**IMPURITIES****Inorganic Impurities**• **LEAD, Lead Limit Test, Appendix IIB**

**Sample solution:** Prepare as directed for organic compounds.

**Control:** 5  $\mu$ g Pb (5 mL of *Diluted Standard Lead Solution*)

**Acceptance criterion:** NMT 5 mg/kg

**SPECIFIC TESTS**• **LOSS ON DRYING, Appendix IIC (105° for 3 h)**

**Acceptance criterion:** NMT 0.3%

• **OPTICAL (SPECIFIC) ROTATION, Appendix IIB**

**Sample:** 8 g, previously dried

**Analysis:** Dissolve the *Sample* in sufficient 6 N hydrochloric acid to make 100 mL.

**Acceptance criteria:**

$[\alpha]_D^{20}$ : Between +21.3° and +23.5°, on the dried basis; or

$[\alpha]_D^{25}$ : Between +21.3° and +23.4°, on the dried basis.

• **RESIDUE ON IGNITION (SULFATED ASH), Appendix IIC**

**Sample:** 1 g

**Acceptance criterion:** NMT 0.1%

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**Wolf-tec,** <sup>inc.</sup>

20 Kiefer Lane  
Kingston, New York 12401  
USA

Phone: (845) 340-9727  
Fax: (845) 340-9732  
**24 Service Hour Hotline: 1-800-257-4627 ext.162**

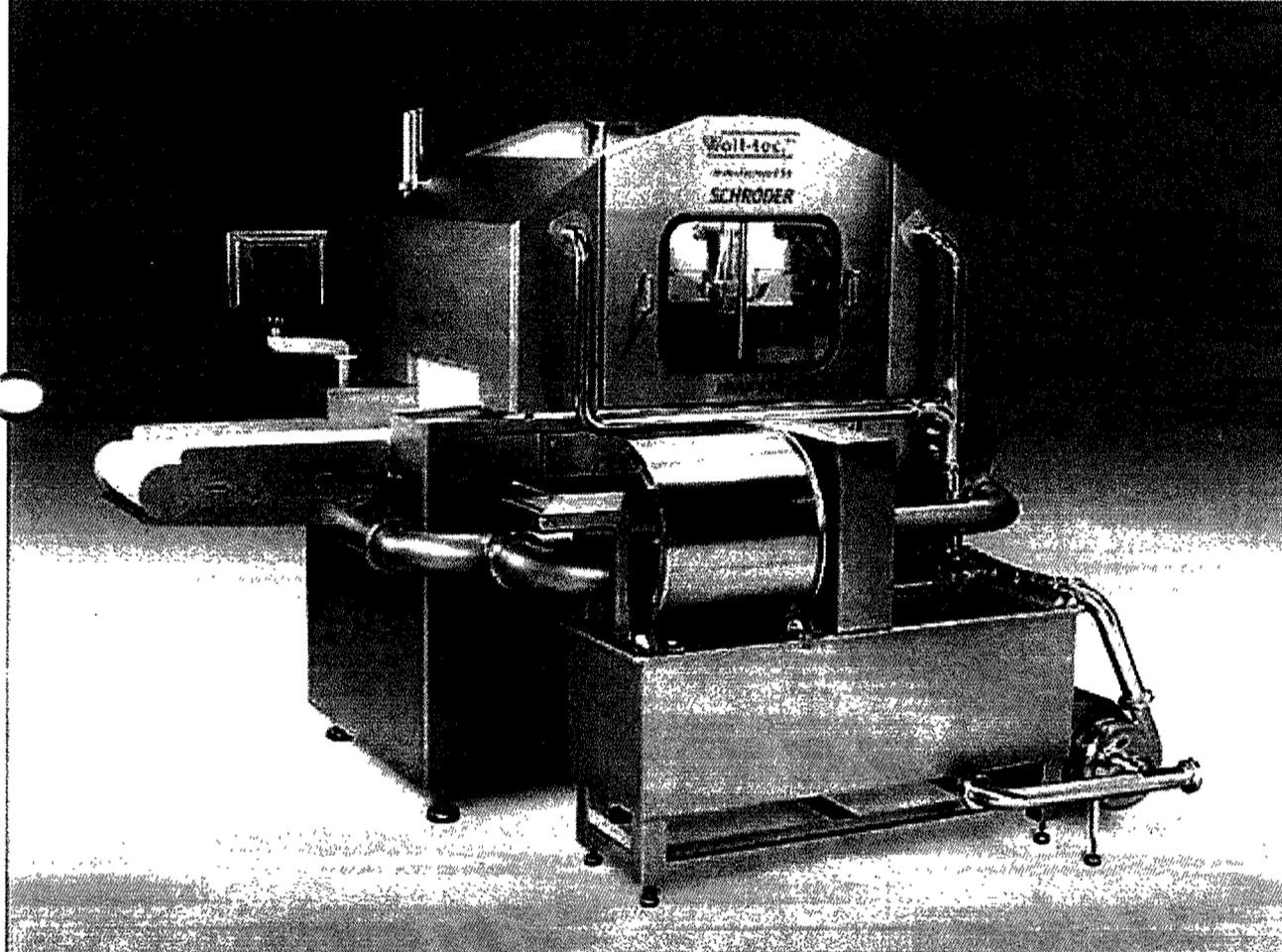
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Date: 05.01.2009

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## Dissolver Slideshow

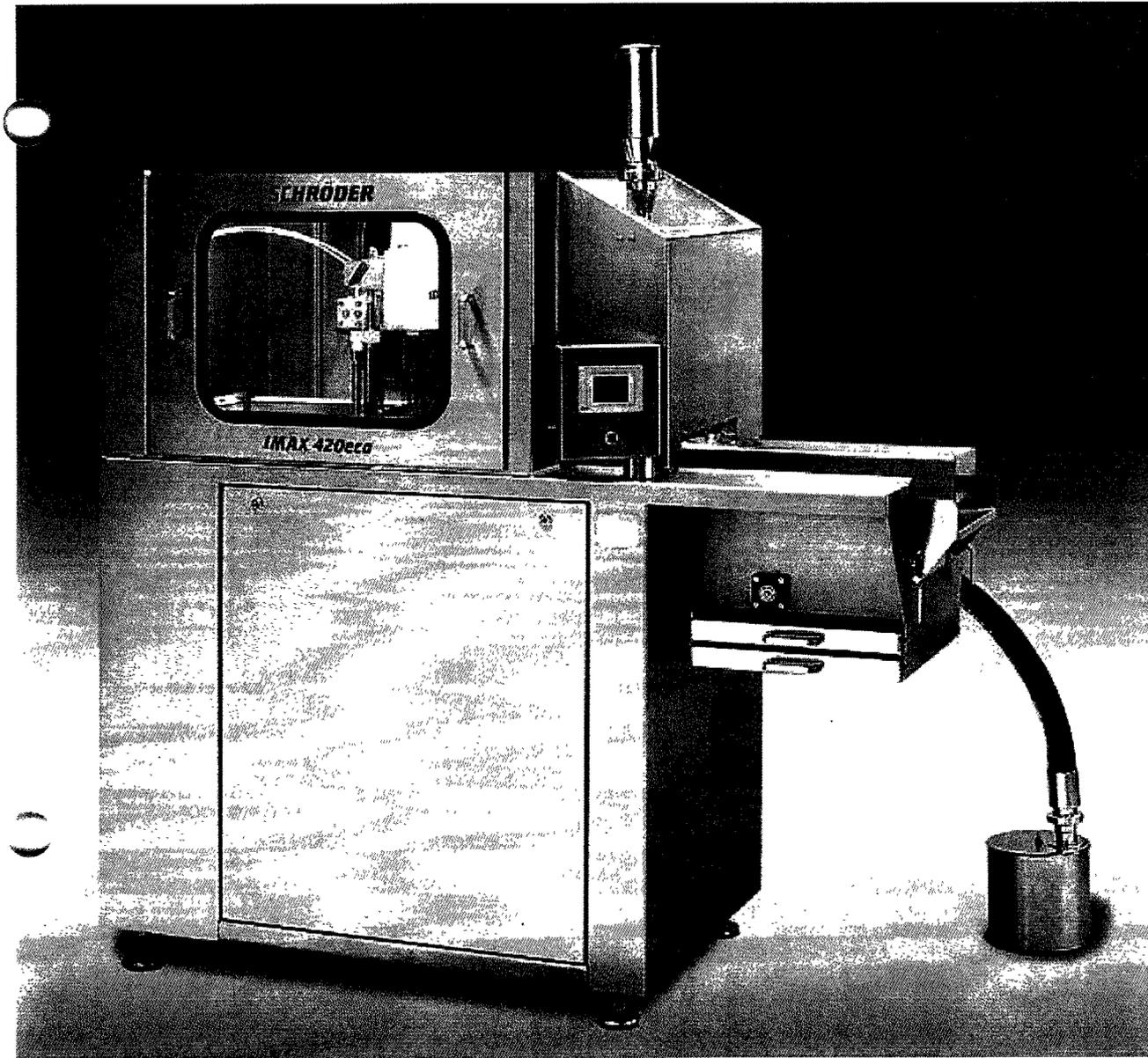
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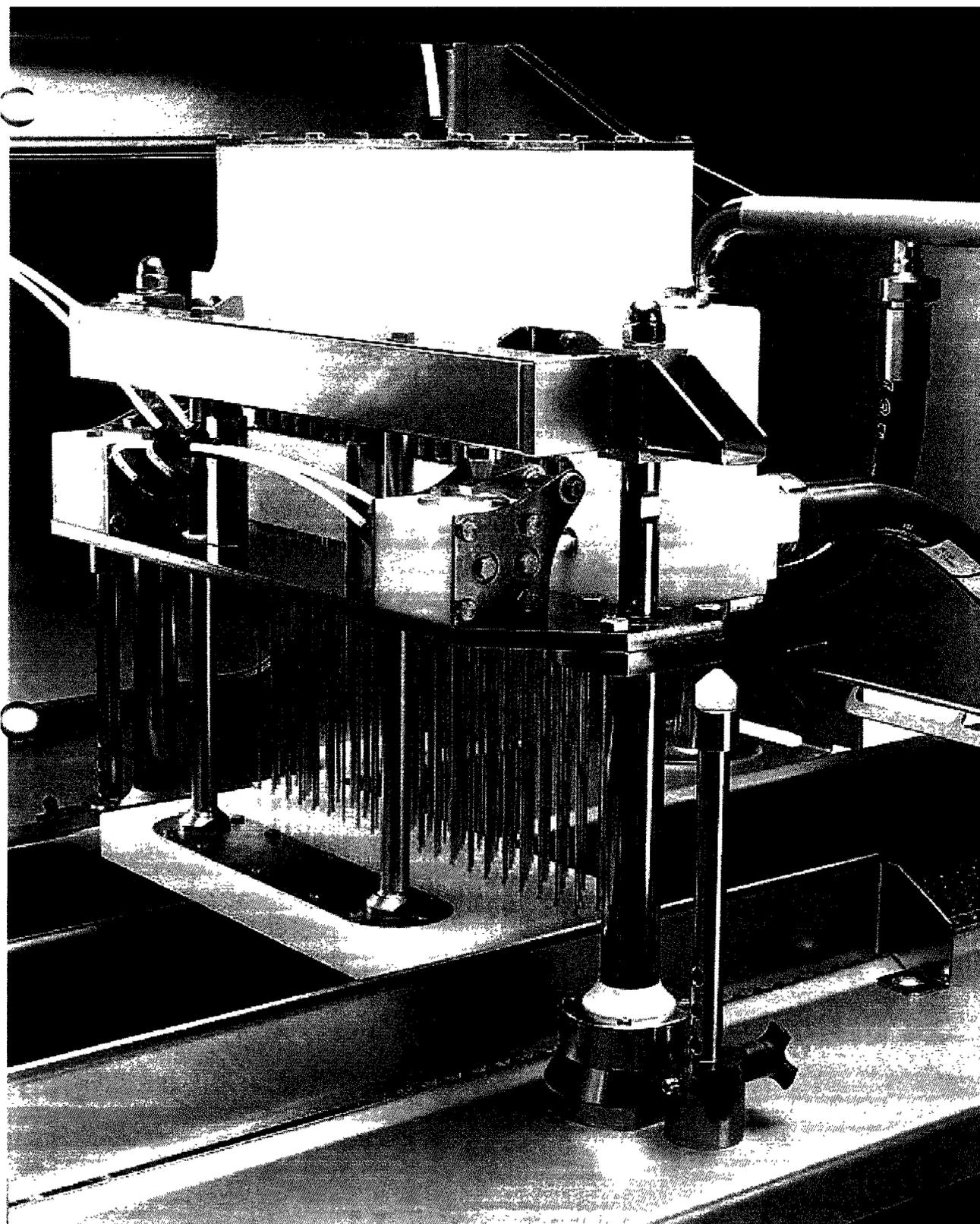
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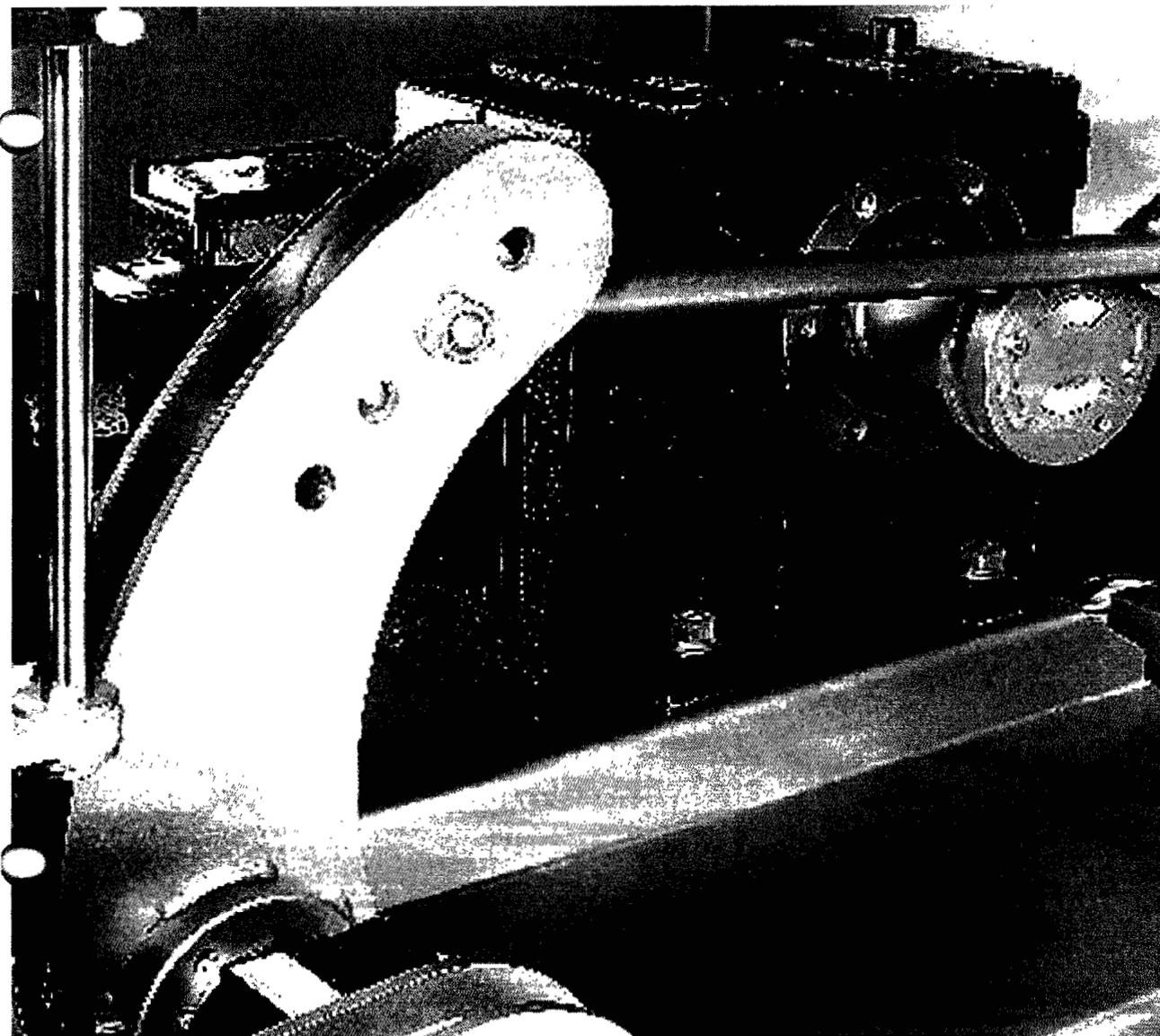
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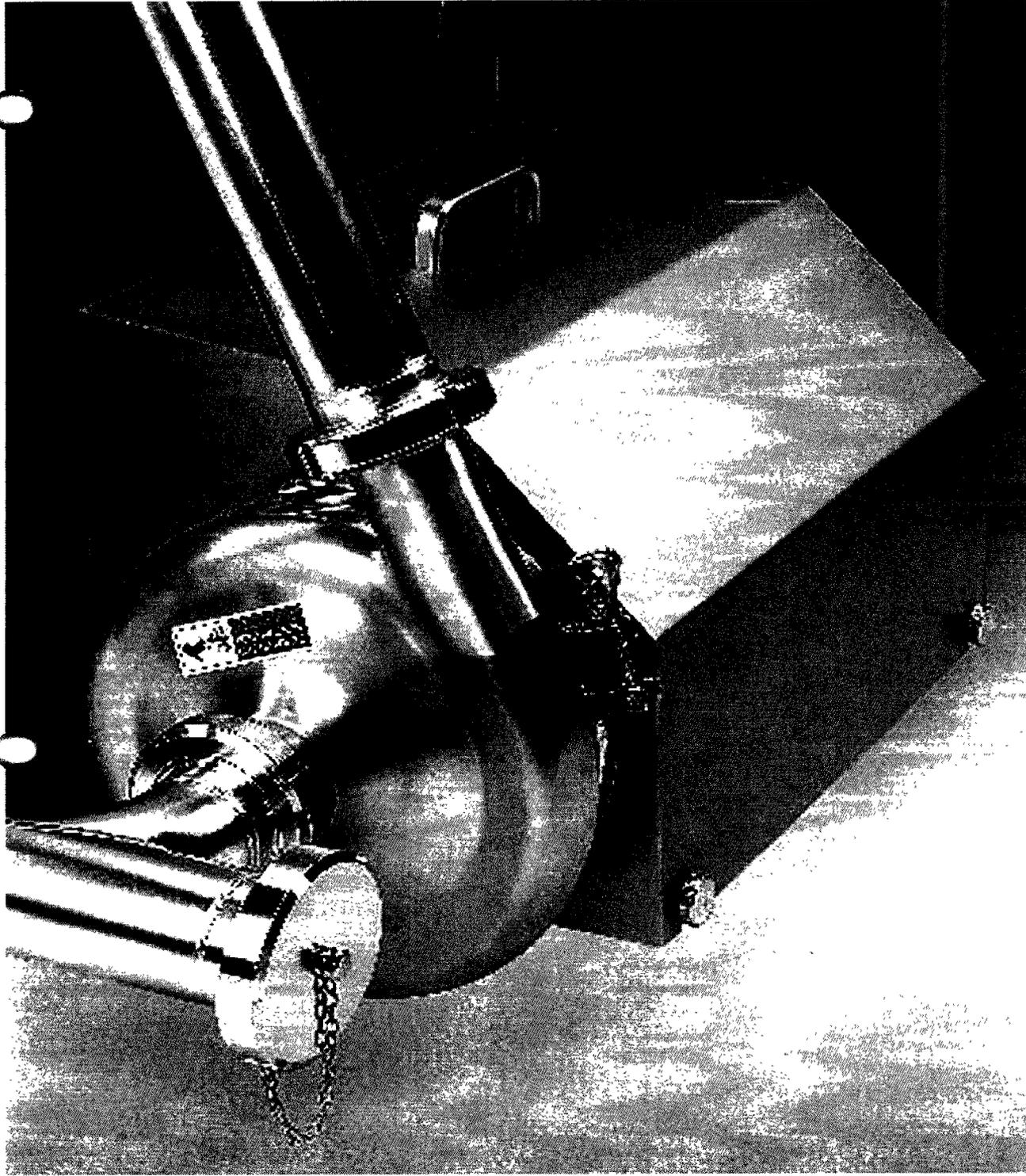
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Pages 000033 - 000054 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

SUBMISSION END

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## *Reference List for Industry Submission, GRN 000290*

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000033 - 000054	Shao, Andrew; Hathcock, John N.	Risk assessment for the amino acids taurine, L- glutamine and L-arginine	2008	Regulatory Toxicology and Pharmacology	Volume 50, pgs 376-399

*NA- Not applicable*

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