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July 15, 1985

Document Control Officer
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Ladies and Gentlemen:

BASF Wyandotte Corporation (BWC) is submitting this notice in accordance with TSCA Sec. 8(e) on the chemical 1-Ethenyl-2-pyrrolidinone, CAS Reg. No. 88-12-0. Enclosed is a copy of the confidential submission and the toxicological studies available to date. BWC is claiming import and sales volumes to be confidential. A sanitized copy of the notice is attached.

If there are any questions regarding this submission, please contact me at (313) 246-6207.

Sincerely,

BASF WYANDOTTE CORPORATION

Edward J. Kerfoot

Edward J. Kerfoot, Ph.D.
Director, Toxicology and
Industrial Hygiene

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SANITIZED COPY

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July 15, 1985

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U. S. Environmental Protection Agency
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Washington, D.C. 20460

Reference: Notice in Accordance with Section 8(e)

Gentlemen:

This notice is submitted in order to provide information on a potential substantial risk associated with 1-Ethenyl-2-pyrrolidinone CAS Reg. No. 88-12-0 which can be further described as follows:

Chemical Formula:	C_6H_9NO
Molecular Weight:	111.1
Physical State:	Colorless Liquid

Toxicological information on 1-Ethenyl-2-pyrrolidinone were received on July 1, 1985 from our ultimate parent company BASF Aktiengesellschaft in Ludwigshafen, West Germany. In one of the preliminary studies, neoplasms in the liver of female rats were observed at hepatotoxic concentrations. While these effects were only found in the treated animals, we wish to emphasize that this study was not designed as a carcinogenicity study, and therefore it would be inappropriate to draw any conclusion concerning the carcinogenic property of 1-Ethenyl-2-pyrrolidinone from these limited data. In spite of the limited nature of these data, we are nevertheless making this submission under TSCA Section 8(e).

1-Ethenyl-2-pyrrolidinone is manufactured by BASF Aktiengesellschaft (BASF AG) and imported by BASF Wyandotte Corporation.

1-Ethenyl-2-pyrrolidinone is used as a raw material for the manufacture of a variety of chemical products.

In 1980, BASF AG initiated a toxicology program on 1-Ethenyl-2-pyrrolidinone. The several studies conducted are listed in Attachment #1. At first a three-month inhalation study was conducted in rats at 0, 5, 15, 45, and 120 ppm with 10 animals sex/group [Attachment #1, reference (1)]. 120 ppm was lethal for the rats. The liver and the olfactory mucosa of the nasal cavity were the target organs. The severity of these changes was concentration-dependent with minimal changes at 15 ppm in the liver and 5 ppm in the nasal mucosa. A subsequent study established a no-effect level of 1 ppm [Attachment #1, reference (2)].

Because of the observation of liver toxicity in the first study, in March 1983 BASF AG initiated a preliminary study to investigate the rat liver's recovery from this toxicity [Attachment #1, reference (3)]. In this study, female rats were exposed to 45 ppm for three months and sacrificed at 7 weeks (during treatment), 3 months (end of treatment), 12 and 24 months. The draft interim reports show that there was evidence of hepatic toxicity at 7 weeks which was more marked at three months. At the interim kill at 12 months, there was marked recovery of liver architecture and recovery from toxicity. Of the 15 animals, each designated for kill at 24 months, 6 of the treated group and 4 from the controls survived this period. There appeared to be no difference in mortality between control and treated. Of the animals killed at the termination, 2 possible carcinomas were observed, and 2 other animals in the treated group had nodular lesions originally reported as "neoplastic nodules". In the control group one animal showed a nodular lesion similar to the neoplastic nodules in the treated group (see report of W. Butler, Attachment #2). None of the animals in the treated group dying or killed prior to termination showed evidence of carcinoma or neoplastic nodules.

Additional toxicity testing conducted by BASF AG includes a three-month inhalation test in hamsters [Attachment #1, reference (4)], as well as a 3 week and a 3 month drinking water studies in rats and a 3-month gavage study in rats [Attachment #1, references (5) and (6)]. These studies confirmed the hepatotoxicity of 1-Ethenyl-2-pyrrolidinone to the rat; however, the compound is not toxic to the hamster at the concentration tested.

Mutagenicity testing in four in-vitro systems [Ames, mouse-lymphoma, cell-transformation, UDS in rat hepatocytes, Attachment #3] has confirmed that 1-Ethenyl-2-pyrrolidinone is not genotoxic.

The data developed to date by BASF AG is not conclusive to establish that 1-Ethenyl-2-pyrrolidinone presents an unreasonable risk of injury to health. Nevertheless, BASF AG is continuing

its toxicology program by conducting in vivo DNA-binding, as a next step to extend the basis for the non-genotoxicity of the compound. However, further metabolism and teratogenicity studies as well as long-term studies are considered necessary in order to develop more conclusive data on the toxicological profile of 1-Ethenyl-2-pyrrolidinone.

In addition, BASF Wyandotte Corporation will notify its customers. Based on the information available to date, there appears to be no health hazard to workers when the recommended precautions are taken to minimize exposure.

As stated in Attachment (1), the enclosed reports are drafts designated for preliminary information only. Final reports and additional translations concerning the referenced studies will be submitted to EPA. Representatives of BASF AG and BASF Wyandotte Corp. would be pleased to meet with EPA to discuss any aspect of this submission.

Very truly yours,

BASF WYANDOTTE CORPORATION

G. W. Paul
Vice President
Research and Development

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Attachments

List of Studies

Re: 1-Ethenyl-2-pyrrolidinone
CAS Registry No. 88-12-0

Enclosed are copies of drafts or draft interim reports for the following 1-Ethenyl-2-pyrrolidinone studies (CAS Reg. No. 88-12-0):

(1) Study on the subchronic inhalation toxicity of N-vinylpyrrolidone-2 in Sprague-Dawley rats (three-month study); Project No. 42I0165/8005 (September 3-October 19, 1980)

(2) Supplementary study on the subchronic inhalation toxicity of N-vinylpyrrolidone-2 in Sprague-Dawley rats (three-month study); Project No. 42I0186/8233 (March 10-June 27, 1983)

(3) Preliminary study on the reversibility of the subchronic inhalation toxicity of N-vinylpyrrolidone-2 in female Sprague-Dawley rats (three-month exposure and subsequent recovery periods of 3, 9, and 21 months); Project No. 51I0186/8234 (March 10, 1983-March 18, 1985)

(4) Preliminary study on the subchronic inhalation toxicity of N-vinylpyrrolidone-2 in Syrian hamsters (three-month study); Project No. 52I0186/8235 (March 14-June 23, 1985)

(5) Preliminary studies on the toxicity of N-vinylpyrrolidone-2 after administration via drinking water over 3 or 4 weeks respectively and by gavage over 13 weeks to Wistar rats; Project No. 12C0053/8404 (February 9-June 23, 1984)

(6) Study on the subchronic toxicity of N-vinylpyrrolidone-2 after administration via drinking water over 13 weeks to Wistar rats; Project No. 32C0250/8417 (July 25-November 6, 1984)

Experimental design and data from these studies are listed on the following table.

Investigations with 1-Ethenyl-2-Pyrrolidinone

Conducting facility for all studies: Department of Toxicology, BASF

ref. No.	species/strain	exposure route/ duration	No. of animals male/female	dose/conc.	time schedule	investigations performed Clinical Chemistry and Hematology	Histopathology
(1)	Sprague-Dawley rat/Caw Ico	inhalation/ 3 months	20/20 10/10 10/10 10/10 10/10	0 ppm 5 ppm 15 ppm 45 ppm 120 ppm	Sept. 3 - Oct. 19, 1980	Blood Chemistry Enzymes Hematology Electrophoresis Urinalysis	29 organs
(2)	Sprague-Dawley rat/Caw Ico	inhalation/ 3 months	10/10 10/10	0 ppm 1 ppm	March 10 - June 27, 1983	Blood Chemistry Electrophoresis	liver nasal cavity
(3)	Sprague-Dawley rat/Caw Ico	inhalation/ 3 months			March 10, 1983 - March 18, 1985	γ-GT and glutathione in liver homogenate γ-GT in plasma	liver
		interim kill 7 weeks after beginning	-/5 -/5	0 ppm 45 ppm			
		interim kill 3 months after beginning	-/10 -/10	0 ppm 45 ppm			
		interim kill 12 months after beginning	-/10 -/10	0 ppm 45 ppm			
		terminal kill 24 months after beginning	-/15 -/15	0 ppm 45 ppm			
(4)	syrian hamster Cpb-Sh Ga51	inhalation/ 3 months			March 14 - June 23, 1983		liver
		interim kill 7 weeks after beginning	5/5 5/5	0 ppm 45 ppm		none	
		interim kill 3 months after beginning	10/10 10/10	0 ppm 45 ppm		γ-GT and glutathione in liver homogenate γ-GT in plasma	
(5)	Wistar-rat/ Chbb-THOM	drinking water 3 - 4 weeks	5/5 5/5 5/5 5/5 5/5 5/5	0 ppm 200 ppm 400 ppm 700 ppm 1600 ppm 6400 ppm	Feb. 9 - March 8, 1984	none	none
	Wistar-rat/ Chbb-THOM	drinking water 3 weeks	5/5 5/5 5/5 5/5	0 ppm 50 ppm 100 ppm 200 ppm	March 26 - April 16, 1984	Blood Chemistry Enzymes Hematology Electrophoresis γ-GT in plasma	none
	Wistar-rat/ Chbb-THOM	stomach tube 3 months	5/5 5/5 5/5 5/5	0 mg/kg 40 mg/kg 60 mg/kg 100 mg/kg	March 26 - June 23, 1984	Blood Chemistry Enzymes Hematology Electrophoresis Clotting analysis γ-GT in plasma and liver homogenate	liver
(6)	Wistar-rat/ Chbb-THOM	drinking water 3 months	10/10 10/10 10/10 10/10 10/10	0 ppm 5 ppm 12 ppm 30 ppm 75 ppm	July 25 - Nov. 6, 1984	Blood Chemistry Enzymes Hematology Clotting analysis γ-GT in plasma and liver homogenate	35 organs and target organ all gross lesions

Attachment 2

THE BRITISH INDUSTRIAL BIOLOGICAL RESEARCH ASSOCIATION

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00008

REVIEW OF 'STOP' EXPERIMENT ON N-VINYLPYRROLIDONE-2 (NVP)

BY THE INHALATION ROUTE TO THE FEMALE RAT

BASF PROJECT NO. 51J 0186/8234

Date of review 12 June 1985

W.H. Butler, MB, BS, FRCPath, ATS

~~Confidential~~ report to

Mr P. Gierlich
BASF Aktiengesellschaft
Legal Department
6700 Ludwigshafen
Germany

The British Industrial Biological Research Association,
Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK

Sections of liver prepared from the 'stop' inhalation study on N-Vinylpyrrolidone (NVP) (Project No. 51J0 186/8234) were made available to me in the laboratories of B.A.S.F. In this experiment groups of female Sprague-Dawley rats were exposed to 45 ppm NVP by inhalation for 3 months. Following 7 weeks of treatment 5 control and 5 treated rats were killed. At completion of exposure 10 treated and 10 controls were killed. A further group of similar size was killed following 9 months recovery without NVP treatment leaving 15 treated and 15 controls for planned killing following 21 months recovery giving a total duration of 24 months.

Of this group of 15 rats 4 controls and 6 treated survived to the terminal kill.

The remaining animals were found dead or killed in extremis there being no difference in the longevity of the two groups.

Of the six animals surviving to terminal kill 1 (107) showed a large nodule within which were focal areas of thickened abnormal trabeculae and necrosis suggestive of hepatocarcinoma. There was no evidence of invasion or metastasis. Another rat (109), as well as large necrotic angiosarcoma of liver, had a large simple nodule with persistant portal tracts. There was not sufficient evidence to consider this lesion neoplastic. Two further animals had multiple large nodules with residual normal structures indicative of hyperplasia.

Of the animals dying or killed prior to the terminal kill 4

survived longer than a total of 18 months and 1 showed evidence of basophilic foci.

Therefore in the group of 15 rats designated for the terminal kill group, 1 rat with hepatocarcinoma was observed and 3 with simple hyperplastic nodules all occurring at 2 years. In contrast the control group, 1 rat (79) had a small basophilic nodule (hyperplasia) and two showed basophilic foci. An angiosarcoma was also observed in the controls.

Animals were also killed at 7 wks during treatment and at 3 months at the termination of treatment. At 7 weeks there was evidence of extensive hepatotoxicity with small eosinophilic clear cell foci. At 3 months the evidence of hepatotoxicity was more marked with centrilobular hypertrophy and increased eosinophilia. The archetecture of the liver was distorted with many areas of glycogen retention, clear cell foci and basophilic foci.

A striking feature of this preliminary experiment was the findings following 6 months recovery. The degree of hepatotoxic change was markedly reduced. Although the overall lobular and trabecular archetecture was disturbed much of the liver was normal. The centrilobular hypertrophy and eosinophilia was reduced. The glycogen retention areas and eosinophilic and clear cell foci were reduced to an extent that in many of the rats no foci were observed. These findings would indicate that the observations made 7 wks and 3 months are in the main related to the hepatotoxic action of NVP.

Conclusion

NVP is hepatotoxic for the rat liver. A high dose resulted in centrilobular necrosis and biliary proliferation. The concentration used in this experiment (45 ppm) for 3 months induced marked hepatotoxicity. However, the degree of change observed at the terminal kill while being similar to the normal senescent changes in the rat were more marked than would be expected. Based on the limited evidence of this preliminary study in the rat by the inhalation route a very tentative conclusion can be drawn that the compound (NVP) may possibly result in neoplasia of the liver at hepatotoxic concentrations.

INDIVIDUAL ANIMAL REPORTS

Female rats receiving 45 ppm NVP by inhalation

Animal No.	
	K. 7 wks Liver
81	No evidence focal change
82	Small eosinophilic clear cell foci
83	Small eosinophilic clear cell foci
84	Small eosinophilic clear cell foci
85	Small eosinophilic clear cell foci
	K. 3 months
86	Mult. large foci both eosinophilic and basophilic with compression. Increase centrilobular eosinophia.
87	Mult. eosinophilic and basophilic foci
88	Occassional eosinophilic foci
89	Increase centrilobular eosinophilic and hypertrophy basophilic foci
90	Mult. eosinophilic and basophilic foci
91	Centrolobular eosinophilia and hypertrophy, basophilic foci
92	Centrilobular eosinophilia and hypertrophy and eosinophilic focal change
93	Centrilobular eosinphilia and hypertrophy
94	Centrilobular eosinophilia with clear cell foci. One basophilic focus
95	Centrilobular eosinophilia and hypertrophy

Animal No.		
	3 months exposure plus 6 months recovery	
96		NAD
97		NAD
98		Occasional small eosinophilic foci
99		Some areas of distorted lobular archetecture observed NAD
100		Multiple diffuse areas of glycogen retention
101		Some disturbance lobular arechetecture with occasional clear cell focus
102		Some distortion lobular archetecture no evidence foci
103		Some disturbance lobular archetecture with residual centrilobular eosinophilia
104		Individual clear cell foci
	3 months exposure followed by 21 months recovery	
106	K.M. 23.5 months	Mult. basophilic foci
107	T.K. 24 months	Large nodule with areas of abnormal trabeculae and necrosis suggestive of hepatocarcinoma
108	T.K. 24 months	Mult. nodules with fatty degeneration and compression
109	T.K. 24 months	Large haem. mass with necrosis. Mult. vascular spaces. Angiosarcoma. Large simple nodule with portal tracts and central veins. No evidence of carcinoma
110	F.D. 16 months	Autolysis bile duct proliferation

Animal No.

3 months exposure followed by 21 months recovery

111	K.M. 20 months	Centrilobular fatty change no evidence nodule
112	K.M. 17 months	Occasional clear cell and basophilic focus
113	K.M. 15 months	Occasional clear cell focus
114	T.K. 24 months	Small basophilic focus
115	F.D. 19 months	Autolysis no evidence nodules of foci
116	K.M. 17 months	No evidence nodules or foci
117	T.K. 24 months	Large simple nodule with portal tracts and central veins - Hyperplasia. Also multiple small foci and eosinophilic nodules
118	F.D. 22 months	Extensive autolysis no evidence nodules in foci
119	K.M. 22 months	Focal fatty infiltration disturbing lobular pattern
120	T.K. 24 months	Multiple clear cell foci and basophilic nodules

Control rats for 24 months survival

66	F.D. 23 months	Autolysis
67	K.M. 20 months	NAD
68	K.M. 24 months	Infiltration with lymphoma
69	F.D. 12 months	Autolysis
70	T.K. 24 months	NAD
71	T.K. 24 months	Biliary proliferation otherwise NAD
72	K.M. 20 months	NAD
73	K.M. 22 months	Biliary cystadenoma

Animal No.

Control rats for 24 months survival

74	T.K. 24 months	Occasional small basophilic focus
75	K.M. 22 months	Occasional basophilic focus and distortion lobular pattern
76	F.D. 18 months	Autolysis
77	T.K. 24 months	Biliary proliferation otherwise NAD
78	K.M. 22 months	Anoplastic sarcoma - Angio sarcoma
79	K.M. 19 months	Small basophilic nodule with compression
80	K.M. 8 months	NAD

Attachment 3

Mutagenicity tests with N-vinylpyrrolidon

Test system	dose/ conc.	time schedule	conducting facility
Ames test			
S. typhimurium TA 98, 100, 1537 with and without S-9 mix	31.5 - 3000 µg/plate	May, 1978	Institute of Biochemical Pharmacology, Mainz, FRG
Ames test			
S. typhimurium TA 98, 100, 1537, 1535 with and without S-9 mix	10 - 10,000 µg/plate	April, 1978	Huntingdon Research Centre, England
in vitro transformation of BALB/ 3T3 cells	0.000061 - 1,000 µl/ml	January, 1980	Litton Bionetics, USA
Mouse Lymphoma forward mutation assay with and without S-9 mix	0.313 - 5,000 µl/ml	January, 1980	Litton Bionetics, USA
Rat hepatocyte unscheduled DNA synthesis	0.284 - 18.2 µl/ml	January, 1980	Litton Bionetics, USA

Aus der Abteilung Toxikologie der
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25698n 5M 7107 (22/20441)

BASF
Gewerbehygiene und
Toxikologie

kli-br

DRAFT

BAND I

Prüfung der subchronischen Inhalationstoxizität von

N-Vinylpyrrolidon-2

an Sprague-Dawley-Ratten
(3-Monate-Versuch)

Berichtsteil

Dieser Bericht ist Eigentum der BASF Aktiengesellschaft und darf nur mit deren ausdrücklicher Genehmigung weitergegeben, vervielfältigt und veröffentlicht werden.

25698n 5M 7107 (22/20441)

(00021)

TRANSLATION

1. SUMMARY

In this test, the tolerability of N-vinyl-2-pyrrolidone (NVP) on repeated intake by inhalation was tested. Vapors of the product, at concentrations of 120 ppm, 45 ppm, 15 ppm and 5 ppm, were offered to Sprague-Dawley rats for inhalation for 6 hours each working day over a period of 3 months. Two control groups were exposed to fresh air over the same period.

16 of 20 animals in the 120 ppm test group died by or during the 5th exposure. 4 animals were sacrificed in a very moribund state after the 5th exposure.

The picture of findings on the animals at the highest concentration of 120 ppm showed clear signs of toxicity: irritant effects on the mucous membranes, apathy, atony and cyanosis. In accordance with the hematuria observed in a few animals, there were indications in the gross-pathological examination of generalized hemorrhagic diathesis, especially of the mucous membranes of the stomach and forestomach. In addition, most of the animals were found to be cachectic on necropsy, and the histopathology showed regressive fatty degeneration of the liver with transitions to toxic dystrophy, together with deeply penetrating necroses in the olfactory epithelium of the nasal cavity.

None of the animals in the test groups exposed to 0, 5, 15 or 45 ppm died during the period of the test, nor did any show clinical signs. Up to a concentration of 15 ppm there was a concentration-dependent effect of the test substance on the animals which took the form of a retardation of body weight gain and a reduced consumption of food. These effects were not detectable at 5 ppm.

The principal clinicochemical changes detected comprised a decrease, which was related to the concentration, in the figures for total protein, albumin and, in particular, α_1-globulin. This effect of the test substance was more pronounced in the female than in the male rats, and was generally more pronounced in the blood samples taken in the middle of the test period than at its end. At the lowest concentration of 5 ppm, these figures were changed significantly only in the female animals. A decrease in the figures for creatinine, urea and potassium was again more pronounced in the female animals. Significant changes in the creatinine and urea figures in the plasma of the female rats were detectable in the second half of the test period down to the lowest concentration of 5 ppm. The changes were concentration-dependent.

In contrast, increases in the total bilirubin content and the alkaline phosphatase activity and a decrease in the cholesterol level were more pronounced in the male animals up to 15 ppm than in the females, in which the figures were found to be changed only at 45 ppm.

In the red blood picture, both sexes in the 15 and 45 ppm test groups showed very slight decreases in the figures for the hemoglobin, hematocrit and erythrocytes, and a reduction in the mean cell volume and slightly raised platelet counts. In the white blood picture of the same test groups, a marginal increase in the leucocyte counts (inflammatory processes) was detected in a few animals.

With the exception of the absolute liver weights of the male animals in the 15 ppm test group, the absolute and relative liver weights were very high in both sexes in the 15 ppm and 45 ppm test groups.

The animals in the 15 ppm and 45 ppm test groups were found to have centro-acinar enlargement of hepatocytes (hypertrophy) together with foci, with distinct boundaries, of atypical hepatocytes, whose morphology substantially resembled the "foci and areas of cellular alteration" described in the British and American literature.

Inflammatory processes (rhinitis) were detected in the nasal mucous membranes in all test groups which received administration of the test substance. The olfactory epithelium in the test groups exposed to 5 ppm - 45 ppm showed concentration-dependent changes in the form of epithelial flattening (atrophy), and the respiratory epithelium showed concentration-dependent changes in the form of hyperplasia.

Taking all the findings into account in the assessment results in the following picture:

Under the chosen test conditions, multiple inhalation of N-vinyl-2-pyrrolidone by rats caused, at the highest concentration (120 ppm), severe liver changes, necroses of the nasal mucosa and a generalized hemorrhagic diathesis, and led to the death of most of the animals within 5 days. At lower concentrations, there were also changes to the liver (15-45 ppm) and nasal mucosa (5-45 ppm). The histopathology showed both centro-acinar hypertrophy of hepatocytes and the occurrence of focal alteration of

hepatocytes. Changes in the plasma proteins (dysproteinemia), total bilirubin, cholesterol and alkaline phosphatase resulting from the liver damage were found. Thus, N-vinyl-2-pyrrolidone appears to cause damage especially in the liver, it being possible for there to be a degree of adaptation with increasing exposure. This is indicated by a diminution in the changes in some plasma figures at the end of the test. The irritant effect of the test substance on the nasal mucous membranes led to inflammatory processes, to atrophy of the olfactory epithelium and to hyperplasia of the respiratory epithelium. Experience with other irritants indicates that these changes in the rat, which breathes through the nose and has a correspondingly active and sensitive nasal mucosa, should be relatively easily reversible.

Under the chosen test conditions, the no adverse effect level for rats was at concentrations $\leq$ 5 ppm. Even at 5 ppm the only changes detectable were those which are typical of animals which breathe through the nose, and are reversible, to the nasal mucosa and, only in the female rats, dysproteinemia and reductions in creatinine, urea and potassium.

A test with a lower concentration (1 ppm) was initiated in order to establish a clear no adverse effect level for N-vinylpyrrolidone in rats on exposure by inhalation.

4.4 Pathology

The results of the test are to be found summarized in the following Tables in the Annexe:

	Table	Page
Key to the abbreviations		
BODY AND ORGAN WEIGHTS		
absolute body and organ weights: - male and female animals in test groups 0, 1 and 2	72 - 73	
relative organ weights: - male and female animals in test groups 0, 1 and 2	74 - 75	
absolute body and organ weights: - male and female animals in test groups 01 and 4	76 - 77	
relative organ weights: - male and female animals in test groups 01 and 4	78 - 79	
GROSS-PATHOLOGICAL FINDINGS		
male and female animals	80 - 83	
HISTOPATHOLOGICAL FINDINGS		
male and female animals	84 - 93	

The individual values and findings are to be found in part C of the Tables (Volume II).

Owing to the premature elimination of test group 3 (120 ppm), in which 7 male and 9 female rats had died after only 4 days of the test, the results of the test can be divided into 2 parts, as follows:

1. "acute" changes after brief exposure to high concentrations of test substance, and

2. "subchronic" findings after inhalation of the test substance for 3 months.

The findings in test groups 0 (control = 0 ppm), 1 (15 ppm) and 2 (45 ppm) belong in the latter category. They are dealt with together with the findings in the test groups 01 (control = 0 ppm) and 4 (5 ppm) which were a later addition to establish a no adverse effect level after the elimination of test group 3 (120 ppm).

Only gross-pathological examination of the animals in the test group at the highest concentration (120 ppm) showed changes which can be attributed to the test substance. The majority of these animals were cachectic, had lost adipose tissue to varying extents and had erosions, some of which were bleeding and some not, and mucosal hemorrhages in the stomach, which in some cases had impregnated the chyme in the small intestine with blood. Thus many of the animals presented the appearance of hypovolemic anemia (Table 80). One animal (No. 40) was found to have a hemorrhagic effusion in the abdominal cavity.

The histopathology of the changes in the stomach showed in some cases profuse bleeding (of the nature of hemorrhagic diathesis) and in some cases bleeding erosions of the glandular stomach mucosa and necroses (ulcers) of the forestomach mucosa with a tendency to calcification (Table 90). Histopathological indications of generalized hemorrhagic diathesis were also found in other organs (e.g. heart, jejunum, thymus, adrenals and brain).

the most noteworthy organic finding, and the cause of the premature death of the animals, was severe liver changes (regressive fatty degeneration, omental necroses and dystrophy) - Tables 87 and 88, which were found by light microscopy in all animals in the test group at the highest concentration and had already been noticed on gross-pathological examination as partially regressive fatty degeneration of the liver parenchyma with transitions to dystropny (Table 81).

Another gross finding related to the test substance in test group 3 was that the adrenals appeared to be enlarged and, depending on the blood content, were sometimes pale and sometimes dark red (Table 81). The histopathological appearance of the adrenal cortex (Table 93) was characterized by hyperemia, small hemorrhages or necrotic processes (multifocal single-cell necroses or dystrophy). Because these animals were not always in a satisfactory state, it was not always possible to distinguish post-mortem from intravital changes, especially in this organ.

The lipid depletion in the cells of the adrenal cortex is interpreted as a non-specific expression of acute intoxication and illustrates the premortal mobilization of the "final reserves". There is a similar relation to the histopathological findings of cell depletion in the spleen, thymus and bone marrow (Tables 91 and 92) and the hyperemia in the major parenchyma (circulatory centralization). They, together with the development of edema in the submucosa of the trachea and the roof of the lung (Tables 86 and 87), are part of the picture of acute shock or the manifestation of a general adaptation syndrome as it was called by SELYE (8). On the other hand, the significance of the yellowish-green discoloration of the adipose tissue cells in the sternal bone marrow, which was found only in the animals in the test group at the highest concentration, remains completely unclear.

Apart from a few spontaneous changes in various organs, nothing which could be attributed to the inhaled test substance was found in the gross pathology of the test animals which were sacrificed after 3 months of the test (Tables 80 to 83).

It emerged from the body and organ weights, which were determined only in groups 0 (0 ppm), 1 (15 ppm), 2 (45 ppm) and the two test groups which were a later addition 01 (0 ppm) and 4 (5 ppm), that both sexes in test group 1 (15 ppm) and above had a highly significant (1% significance level), concentration-dependent reduction in body weight by up to about 16% compared with the controls (Tables 72 and 73). In parallel with this, there were reductions in the absolute heart and brain weights in group 2 (45 ppm), which were more statistically significant for the female animals than for the male animals (1% vs. 5% significance level). An increase in the relative weights of these organs was found at and above 15 ppm (Tables 74 and 75), which appeared to be more pronounced in the male animals than in the female animals and affected both organs in the male rats. In contrast, the female animals showed a slight increase in weight only for the relative brain weight at and above 15 ppm (5% significance level).

The effect on these organ weight parameters may well be caused by the reduction in body weight, since in a catabolic state the amounts of reserves, apart from water, which these two organs can make available are zero (brain) or minimal (heart). Accordingly, nothing relevant was found by light microscopy of these organs.

The same probably applies, in a wider sense, to the changes in the kidney weight. There was a slight statistic reduction (5% significance level) in the absolute weight for the male animals in test group 2. On the other hand, the relative weight was statistically slightly increased (Tables 75 and 79) only in the female rats (5% level of significance) in all the test groups which received administration of the test substance. However, since no clear concentration-effect relation was detectable, the other sex was unaffected in each instance, and there were no gross-pathological or histopathological changes which might explain the increase in weight, the change in the kidney weight does not appear from the viewpoint of pathology to have any particular biological relevance.

The situation was quite different with the liver weights. There was an increase in the absolute weights in the female animals, which was slight (5% significance level) in test group 1 (15 ppm) and pronounced (1% significance level) in test group 2 (45 ppm). For the male rats, the absolute liver weight was slightly raised in test group 2, but there was already a detectable trend in test group 1. There was a great increase (1% significance level) in the relative liver weight in both sexes in these two test groups, the increase being about 32% over the controls for the male animals in the 45 ppm test group and as much as about 40% for the female rats (Tables 74 and 75). The increase in weight was attributable to an enlargement of hepatocytes, which particularly affected the centrolobular areas and was especially pronounced in test groups 2 (45 ppm) and 3 (120 ppm), whereas this was detectable

histopathologically only in one female animal (No. 53) in test group 1 (15 ppm). The etiology and morphology of the enlargement of the hepatocytes in the animals in test groups 1 and 2 differed markedly from that in test group 3. The enlargement of the hepatocytes in the animals in the 120 ppm test group was of the nature of an acute degenerative-toxic cloudy swelling, whereas no signs of degenerative cell damage were detectable on light microscopy in test groups 1 and 2. On the contrary, in the latter there were additional formations, with distinct margins, of enlarged hepatocytes whose cytoplasm was in some cases hydropic and optically virtually transparent and in some cases eosinophilic and with a cloudy structure, and whose nuclei were enlarged, and in some cases hyperchromatic, inside which one or more large nucleoli were detectable. Atypical giant nuclei or bizarre mytoses were found in a few foci. The morphology of these foci was the same as the "foci and areas of cellular alteration" described by STEWART (9) and they were found in all lobule areas (central, intermediate or peripheral) or, especially in the 45 ppm test group, had enlarged beyond the lobules. Both the number and the size were clearly related to the concentration (Table 88).

Since fixation was routinely carried out with aqueous formaldehyde solution, it was not possible to detect glycogen using Best's carmine. However, using the PAS reaction on the formalin-fixed material showed some weakly positive reactions, and these appeared to be more frequent and intense in the hepatocytes in the foci which have been described than in the surrounding parenchyma or in the livers of the control animals. However, it was not possible to quantify the result of the reaction.

Neither enlarged hepatocytes nor this type of "foci" were detected in the control animals in test groups 0 and 01 or in the animals in test group 4 which was a later addition.

It was likewise possible to distinguish between acute and chronic changes in the assessment of the findings on the epithelial tissues in the nasal cavity. The respiratory epithelium of the nasal cavity (levels I and II, Fig. 4.4.1.) and the nasopharyngeal duct (levels III and IV, Fig. 4.4.1) was flattened in many animals in test group 3 (120 ppm), whereas in test groups 4 (5 ppm), 1 (15 ppm) and 2 (45 ppm) this type of epithelium in level I showed hyperplasia whose intensity increased as a function of the concentration (Table 84). There was flattening (atrophy) of the olfactory epithelium at levels II to IV (Tables 84 to 86) in test groups 1, 2, 3 and 4. It had developed over larger areas in the 120 ppm test group than in the other test groups, and there were superimposed necroses of the olfactory epithelium which extended down to the basal cell layer. The latter was found only in the test group exposed to a concentration of 120 ppm of the test substance. There was a detectable increase in the frequency of atrophy with the concentration. Only one animal (No. 53) in the two control groups showed slight focal atrophy of the olfactory epithelium.

The "focal proliferation of the basal cells of the olfactory epithelium" described in levels II to IV is most probably a general repair process. The latter was also found in all 3 levels in the control animals, but there

was no relation between the concentration and the frequency or severity up to the 45 ppm test group. Nothing like this was found in the 120 ppm test group, but this was attributable to the deeply penetrating necroses in the olfactory epithelium and the poor post-mortem state of some of the animals.

Inflammatory reactions of varying severity were found in the various types of mucosa in all 4 levels of section of the nasal cavity in test groups 1, 2, 3 and 4. These were most pronounced in the animals in the 120 ppm test group. Only one control animal (No. 83) had slight rhinitis, and this underlines the fact that these findings were changes related to the test substance.

All the other gross-pathological or histopathological findings listed in Tables 80 to 93 are spontaneous changes or are findings caused by the sacrifice, and they have no evident causal connection with the inhaled test substance.

On the basis of the results of the present investigation, it can be said that administration of N-vinyl-2-pyrrolidone by inhalation in concentrations of 5 ppm, 15 ppm, 45 ppm and 120 ppm to male and female Sprague-Dawley rats for 3 months caused inflammatory changes of varying severity in the nasal cavity as well as atrophy of the olfactory epithelium and hyperplasia of the respiratory epithelium. Both the number and the intensity of the changes depended on the concentration. However, based on experience in earlier tests on other test substances, it may be assumed that both the inflammatory changes and the mild forms of atrophy or hyperplasia will be relatively rapidly reversed. With a sufficiently long observation period, complete recovery of even severe forms of basal cell hyperplasia of the respiratory epithelium is not ruled out.

Apart from the toxic findings, which led to the premature death of virtually all the animals in the 120 ppm test group, changes in the liver related to the test substance occurred at and above a concentration of 15 ppm. They became manifest in some cases as enlargement of hepatocytes (hypertrophy), which was also evident from an increase in weight of the liver, and in some cases as foci or areas of cellular alteration, whose morphology when stained with hematoxylin and eosin was the same as those described by STEWART (9).

From the viewpoint of pathology, the no adverse effect level in this test was at a concentration of test substance below 5 ppm.

TRANSLATION

SUMMARY OF THE HISTOPATHOLOGICAL FINDINGS TABLE: 087

IDENT. NO.: 8005-NVP
TEST SUBSTANCE NO: 80/165
TYPE OF TEST: 3-month inhalation

SPECIES: Albino rat
SEX: male/female

	CODE NO.	GROUP 0 0 ppm		GROUP 0 1* 0 ppm		GROUP 4* 5 ppm		GROUP 1 15 ppm		GROUP 2 45 ppm		GROUP 3 120 ppm	
		10 m	10 f	10 m	10 f	10 m	10 f	10 m	10 f	10 m	10 f	10 m	10 f
Lung:													
- acute emphysema, especially in the apices of the lobes	3430	0	0	0	0	0	0	0	0	0	1	0	2**
- hyperemia, NOS	3810	0	0	1	0	0	0	0	0	0	0	6**	7**
- inflammatory edema in the submucosa and adventitia of the root of the lung	4140	0	0	0	0	0	0	0	0	0	0	2**	6
- chronic bronchopneumonia	4310	0	2	2	0	0	0	0	0	0	1	1	0
Liver:													
- cyst, NOS	3540	0	0	0	0	0	0	0	0	0	0	1	0
- purulent-necrotic inflammation	4070	0	0	1	0	0	0	0	0	0	0	0	0
- focal fibrous adhesive perihepatitis	4814	0	0	0	0	0	0	0	2	0	0	0	0
- foci of necrosis with leukocyte infiltration	[illegible]	0	0	1	0	0	0	2	0	0	0	0	0
- yellow atrophy	[illegible]	0	0	0	0	0	0	0	0	0	0	0	1**
- red atrophy	[illegible]	0	0	0	0	0	0	0	0	0	0	6**	5**
- centro-acinar omental necroses with reactive proliferation of Kupffer cells	[illegible]	0	0	0	0	0	0	0	0	0	0	4	4
- fatty degeneration of isolated cells, NOS	[illegible]	3	3	3	2	3	4	3	3	3	2	0	0
- focal fine-droplet fatty degeneration	[illegible]	2	2	4	3	0	3	1	0	6	5	0	0
- peripheral-acinar fine-droplet fatty degeneration	[illegible]	4	2	0	0	0	3	0	0	0	0	0	0

* - groups added later; ** - only few animals which died prematurely

00032

TRANSLATION

SUMMARY OF THE HISTOPATHOLOGICAL FINDINGS TABLE: 088

IDENT. NO.: 8005-NVP SPECIES: Albino rat
TEST SUBSTANCE NO.: 80/165 SEX: male/female
TYPE OF TEST: 3-month inhalation

	CODE NO.	GROUP 0 0 ppm		GROUP 01* 0 ppm		GROUP 4** 5 ppm		GROUP 1 15 ppm		GROUP 2 45 ppm		GROUP 3 120 ppm	
		10 m	10 f	10 m	10 f	10 m	10 f	10 m	10 f	10 m	10 f	10 m	10 f
Liver (continuation):													
- diffuse fine-droplet fatty degeneration which increases towards the periphery	5530	0	0	0	0	0	0	0	0	0	0	10	10
- focal compression atrophy as a consequence of diaphragmatichernia	7101	0	1	0	0	1	0	0	1	0	0	0	0
- centro-acinar hypertrophy of hepatocytes, NOS	7201	0	0	0	0	0	0	0	1	6	10	0	0
- diffuse hypertrophy of hepatocytes which increases towards the center, NOS	7203	0	0	0	0	0	0	0	0	0	0	10	9
- foci and areas of cellular alteration	7303	0	0	0	0	0	0	1	3	9	10	0	0
Kidney:													
- pelvic dilatation, NOS	5410	1	2	2	3	3	3	0	2	1	0	0	0
- a few tubules with cystic dilatation and eosinophilic casts, NOS	5201	0	1	1	1	0	1	2	0	0	0	0	0
- cyst, NOS	5430	0	1	1	0	0	0	0	0	0	0	0	0
- hyperemia, NOS	5500	0	0	0	0	0	0	0	0	0	0	5**	5**
- focal chronic pyelonephritis	5102	0	1	0	1	0	0	0	0	0	0	0	0
- focal chronic pyelitis	5013	0	0	0	0	0	0	0	0	0	0	0	0

* = groups added later; ** = only for animals which died prematurely

000033